BY THE SAME AUTHOR

HANDBOOK OF CHOLERA

AND

CLINICAL LABORATORY METHODS

TEXT BOOK

OF

PATHOLOGY

INCLUDING BACTERIOLOGY, ANIMAL PARASITOLOGY,
LABORATORY METHODS AND LABORATORY
DIAGNOSIS OF DISEASES.

NOT FOR HOME ISSUE
লাইব্রেরীতে বসিয়া পড়িবেন

BY

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"Handbook of Cholera" and "Clinical Laboratory Methods."

THIRD EDITION
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WITH 350 ILLUSTRATIONS AND 8 COLOURED PLATES

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PREFACE TO THE THIRD EDITION

THE second edition of this volume being exhausted in an unexpectedly short time, the third edition could not follow immediately, as much time had to be devoted for a thorough revision for this new edition.

The whole work has been thoroughly revised, many chapters largely rewritten and many new subjects of importance have been added, *e.g.*, antiquity of Hindu and Greek systems of medicine, filterable viruses and virus diseases, arthropoda, snakes, shock, fever, Hodgkin's disease, diseases of blood, tuberculosis of the lung, pneumothorax, cholera and cholera kidney, peptic ulcers, and such diseases of unknown causation as beriberi, epidemic dropsy and pellagra.

In this edition a great economy of space has been effected by using two different sizes of types and also by putting typescript by the side of the figures. The portions printed in smaller type are intended for those desiring detailed study. Over 50 new illustrations have been added in this edition.

I express my grateful thanks once more to my respected Chief, Prof. Charu Chandra Bose, for his valuable guidance and encouragement and also to Doctors Manindra Nath Dey, Jogesh Chandra Mukherjee, Haran Chandra Banerjee, Nirod Baran Mandal, Hara Prasanna Bhattacharjee, Hemendra Nath Chatterjee, Sushil Kumar Basu and Prabhash Chandra Rakshit for going through the manuscript and proof. I wish also to express my thanks to Doctors Jitendra Nath Maitra, Profulla Kumar Mitra and Nirod Baran Mandal for supplying new pictures for this edition.

Calcutta,
The 1st July, 1932.

DHIRENDRA NATH BANERJEE.

PREFACE TO THE SECOND EDITION

THE second edition of this book is long overdue, the first edition being out of print for more than a year. The very favourable reception of the first edition has been most gratifying.

Its object is the same as that of the first edition. In this edition it has been my endeavour to bring the book up to date. Great advances have been made in our knowledge in recent years and an attempt has been made to introduce this new knowledge without changing to any extent the general arrangement of the book.

Two new chapters have been added, *viz.*, "Evolution of Pathology" and "Incidence of Infections Diseases with special reference to India." Some of the chapters, especially those on disturbances on circulation and metabolism, have entirely been re-written.

Many old illustrations have been omitted and more than eighty new ones introduced. It is a noticeable feature that some of the illustrations are entirely novel, in that this sort of figures appears for the first time in any text-book. These are the total frozen sections as invented by Dr. Christeller, for the study of histo-topography. We are usually accustomed to having a microsection ½"×½" in size, while a size of one inch square is rather unusual. Dr. Christeller has found out a method by means of which the whole organ *e.g.*, the liver, lung, kidney or spleen, can be cut in such a thin slice by frozen method that it can be mounted on a suitable slide (one cleared photographic plate serves best) and after staining by the desired method-hæmatoxylin-eosin, van Gieson, Weigert's elastic, etc.—it can be mounted with Canada balsam as a permanent specimen with a mica foil serving the purpose of a cover glass. These sections are so thin that one can use oil-immersion lens for examination of finer structures. This method, which Christeller terms as histo-topographic method, is a distinct advance in histo-pathology.

I had the privilege of working with Dr. Christeller at Berlin and the figures illustrated in this edition are from specimens prepared by myself at his laboratory.

With a heavy heart we hear the sad news of the death of two of my teachers in Germany, whose names are well-known amongst the scientists of the world, one of them being Dr. Christeller whose death took place at the very early age of 30, and the other Prof. Unna.* This year has taken the toll of yet another scientist—Prof. Widal.

I had the proud privilege of having been the first and at the same time the last one from India to make pilgrimage to Prof. Unna's clinic at Hamburg and work with him.

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* Paul Gerson Unna, the greatest Dermatologist and Histologist of modern times, died in his 79th year in 1929. He was born on 8th September, 1850 in Hamburg. He had his general education at Hamburg after which in 1870 he went to Heidelberg, one of the oldest German Universities, to commence his medical studies. He completed his course of studies at Strassburg where he submitted his thesis for the doctor's degree on the development of the skin. The special feature, which was a novelty at that time, was the staining reaction of the different cellular and tissue elements which form the basis of Unna's work. Von Recklinghausen, his examiner, who upheld a different view, being of the opinion that the staining reactions were only to cloak the power of observation and hence of little importance, objected to the thesis but had to yield subsequently. These obstructions and uncharitable remarks to the young scientist, who became an M.D. when he was only 25 years of age, gave him the stimulus which led to the unparalleled development of the histopathology under the able hands of Unna.

Prof. Unna used to say to the present writer that it was the objections of Von Recklinghausen to accommodate his thesis that had stimulated him to follow this path, so as to show to the world at large the importance and utility of staining reaction in scientific research.

Unna visited numerous foreign countries and so came in direct touch with the leading dermatologists of the time notably Hansen of Norway with whom he worked on leprosy and our present knowledge on histo-pathology of leprosy is mainly due to him. He met Sir Malcolm Morris and published in collaboration with him the *International Atlas of Rare Diseases.* He thus gained the highest esteem and appreciation from all the countries, and was invited almost every year to all the leading associations of the world

He is the father of numerous specific staining reagents for different tissue elements. In 1890 he first described the *plasma cells* which are called after him. He introduced icthyol, Unna's paste and other therapeutic agents for the treatment of skin diseases.

His name is at the top as the author of scientific papers published not only in number, but without exception, of epoch-making importance.

The *British Medical Journal* rightly remarks: "It is no exaggeration to say that Unna has done more than any other single person for the advancement of the speciality to which he devoted his long life, and to which he has been faithful right up to the end."

The *Lancet* further remarks: "With the death of Unna, in his seventy-ninth year, there passes the last of a long series of German and Austrian dermatologists that began with Hebra and Kaposi, and produced such geniuses as Neisser. He had lived so long that his name had become a tradition—a tradi-

The very interesting figures of neurofibroma are taken from the conjoined paper published by Dr. Christeller and myself in Virchow's *Archiv f. Path. Anatomie.*

tion which will last so long in dermatology as the name of Richard Wagnar in music. Between these two men there are other resemblances which render a comparison interesting. Both were highly original, and followed no paths but of their own making. Their work and opinions were unconventional, and both were thus in conflict with the spirit of their times."

During his life he published more than 600 papers in the period of 54 years of which a very large number are of outstanding merit. Of these several are of special interest. These are Histology and development of the human skin and its appendages (published in 1876), Histo-pathology of the Skin (1894), and Histo-chemistry of the Skin (1927). He founded in 1882 and was also the most important and regular contributor of one of the first-class journals of the day—the "Dermatologisches Wochenschrift." In 1897 he published the atlas of the pathological histology of the skin which was completed in 1910 in 9 volumes.

When I went to him he showed me his book "Histo-pathology of Skin" published in 1894, *i.e.*, about 35 years ago and containing 1224 pages As at that time the German language was too difficult for me he helped me with a copy of Norman Walker's translation of that book published in the English language in 1896 I was astonished to see that many chapters on histo-pathology of skin diseases as written by a German as far back as 1894 and existing in English language since 1896 were so exhaustive that even various tropical diseases mentioned in this book have not yet been corroborated in the British Empire or in any other part of the world

For the last 20 years he was working on the subject of chromolysis, by which the chemical constitution of the different component elements of a cell can be very easily determined. For example, the nucleus, the nucleolus, and other cell structures can be specifically stained by Pappenhein-Unna method with pyronin and methyl-green. By using some different dissolving agent, *e.g.*, distilled water, NaCl (2 per cent.), HCl (5 per cent. and 15 per cent.), it is found that some of the component elements have been dissolved by one or the other of these agents, and by staining them again by Pyronin and methyl-green we find these elements missing. From these properties one can determine the chemical composition of these different structures.†

On the wall of his laboratory there was a chart on chromolysis which was the result of 20 years' work of Prof. Unna, which gives us an idea of the laborious task that these pioneers have got to perform, the fruits of which are utilised by us in several hours.

† An idea on this very interesting subject of chromolysis can be obtained from the following articles of the present writer:—

1. Histological determination of the chemistry of cells (chromolysis)—Calcutta Medical Journal, Feb. 1930.
2. Chromolytic determination of the chemistry of Hæmoproteus columbæ—Cal. Med. Jl., April, 1931.

In this edition more than 150 pages of reading matter and over 80 illustrations have been added. All this has added greatly to the cost of pdblication, but in consideration of the well-known poverty of our students, for whom the book is primarily intended, the price has not been enhanced, but has, as before, been kept at Rupees Twelve only.

CALCUTTA,
The 1st June, 1929. DHIRENDRA NATH BANERJEE.

PREFACE TO THE FIRST EDITION

THIS book has been written primarily with the object of publishing a comprehensive text book on pathology and bacteriology intended for the medical students. It is difficult to select a text book for students from the existing ones as no one book includes all the different subjects inseparable from the scope of pathology, namely bacteriology, animal parasitology, laboratory methods, laboratory diagnosis of infectious diseases, transmission problems, pathological explanation of signs and symptoms of diseases. Attempts have been made to cover comprehensively the above subjects, at the same time discussing more fully the pathology of diseases common in this country.

As such, the book will also serve a useful purpose of ready reference for general practitioners.

The plan of the work has been on the following lines. All the diseases have been discussed upon the basis of etiology, laboratory diagnosis, morbid anatomy, morbid histology, morbid physiology and their terminations. No attempt has been made to describe systematically all the diseases occurring in individual organs on anatomical basis, hence there is no division into general and special pathology.

To make the book comprehensive, details of experiments, theories, subject matters of comparatively little interest to students and description of rare and unimportant conditions have been omitted as far as practicable.

Diagnostic methods which are best and suitable for application have been mentioned, leaving aside those which are unimportant or difficult for the students to pick up.

Free use of standard text books and periodicals on pathology, bacteriology and medicine as well as the works on special subjects have been made.

Illustrations—

All the photographs are taken from patients of the Carmichael Medical College Hospital, Pathological Museums of both the Carmichael Medical College and the Calcutta Medical College, and from collections of numerous friends in the profession.

All the microphotographs, skiagrams and several photographs of museum specimens are the work of the author himself. Some of the diagrams will be found to have novel illustrative features.

CALCUTTA,
1st May, 1925.

DHIRENDRA NATH BANERJEE.

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TEXT BOOK OF PATHOLOGY

प्रणम्य जगदुत्पत्तिस्थितिसंहारकारणम् ।
स्वर्गापवर्गयोर्द्वारं त्रैलोक्यशरणं शिवम् ॥
नानामुनीनां वचनैरिदानीं समासतःसद्भिषजां नियोगात् ।
सोपद्रवारिष्टनिदानलिङ्गो निबध्यते रोगविनिश्चयोऽयं ॥
नानातन्त्रविहीनानां भिषजामल्पमेधसाम् ।
सुखं विज्ञातुमातङ्कमयमेव भविष्यति ॥

—Madhaba Kara, in his *Nidanam.*

CHAPTER I

EVOLUTION OF PATHOLOGY

INDIAN SCHOOL

THE evolution of the Indian School of pathology may be arbitrarily divided into four periods—the earliest or the Vedic period, the second or the period of *Samhitas* or original works, the third or the period of compilations, and the last or the period of degeneration.

The earliest or the Vedic period, which is regarded as the dawn of the Ayurveda, exists from eternity, as indicated in the following passage from *Charaka Samhita,* I. 30, 13 सोऽयमायुर्वेदः शाश्वतोनिर्दिश्यतेऽनादित्वात्स्वभावसंसिद्धलक्षणत्वात्भावस्वभावनित्यत्वाच्च । Ayurveda is a part of *Atharva Veda,* the fourth Veda, while by others (as in *Brahmabaibarta Purana*) it has been regarded as the fifth Veda. The date of this period is a puzzle and has occupied the best brains of Oriental as well as Occidental scholars during the last century, but it still remains unsolved. However, it is accepted that it falls in the pre-historic period and that the Vedas are the most ancient literature of the world. It is needless to discuss the evolution of pathology at this remote period of world's history, as that will bring only speculation into play.

The second period, or the period of *Samhitas* or original works, is also of a remote origin, the antiquity of which often baffles our imagination. The greatest works, the redacted editions of which are still extant, are the *Agnivesa Samhita* (commonly known as the *Charaka Samhita* after the name of the redactor **Charaka**) and the *Susruta Samhita.*

"Hessler, in his Latin translation of *Susruta Samhita,* assigned the appearance of this work to a remote period of Indian history, the beginning of which is lost in the immensity of time, and the end of which is known to be about 1000 B.C."—(Mukhopadhayay's *History of Indian Medicine,* Vol. III, p 578) Hœrnle and others are also of the same opinion.—(*Studies in the Medicine of Ancient India,* Part I; *Osteology,* Introduction, p. 9).

Bharadvaja, the son of Vrhaspati, and **Atreya,** the son of the sage Atri, are the two earliest Indian medical practitioners. Atreya had six prominent pupils named **Agnivesa, Vela, Jatukarna, Parasara, Harita** and **Ksharapani,** each of whom has written a compendium of his teachings, the best of which is the *Agnivesa Samhita.* Like the other ones, this Samhita was so disorganised that at the time of **Charaka** (2nd century A.D.) it had to be redacted and re-edited, which work was done by the able hand of Charaka and is still available as the most standard authority of the Ayurvedic system of medicine, although the redacted edition by Charaka was further redacted at a subsequent period by **Dridhabala** (4th century A.D) The *Agnivesa Samhita* thus redacted is now known at the celebrated *Charaka Samhita.*

Susruta, the son of the great sage Viswamitra, learnt *Salya Tantra* or Major Surgery from **Dhanvantari** and wrote a compendium, *Susruta Samhita,* principally dealing with surgical matters.

Even at this remote period the subject of pathology was held to be of great importance, as is seen from the fact that, in both *Charaka Samhita* and *Susruta Samhita,** a separate division consisting of several chapters has been

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* The *Charaka Samhita* consists of eight sections, which are-

सूत्रस्थानम्	General, 30 chapters	इन्द्रियस्थानम्	Senses, 11 chapters
निदानस्थानम्	Pathology, 8 chapters	चिकित्सास्थानम्	Therapeutics, 30 chap.
विमानस्थानम्	Physiology, 8 chapters	कल्पस्थानम्	Pharmacology, 12 chap.
शारीरस्थानम्	Anatomy, 8 chapters	सिद्धिस्थानम्	Purification, 12 chapters

—a total of 119 chapters.

The *Susruta Samhita* consists of six principal sections, which are-

सूत्रस्थानम्	General, 46 chapters	चिकित्सितस्थानम्	Therapeutics, 40 chap.
निदानस्थानम्	Pathology, 16 chapters	कल्पस्थानम्	Pharmacology, 8 chap.
शारीरस्थानम्	Anatomy, 10 chapters	उत्तरतन्त्रम्	Systemic Surgery, 66 chapters

—a total of 186 chapters.

reserved for the subject of pathology. Thus in the *Nidanasthanam,* * or the section on pathology, Charaka deals with this subject in eight chapters. In the *Susruta Samhita,* which consists of six divisions, the second one, consisting of 16 chapters, is devoted to pathology (mainly dealing with etiology and symptoms) of surgical diseases.

From a perusal of these two monumental works it is evident how specialisation originated in two schools like those of our own days—the school of Physicians (*Agnivesa School,* or—after his preceptor—*Atreya School*) and the School of Surgeons (*Susruta School,* or—after his preceptor—*Dhanvantari School*). Not only that, they also divided the Ayurvedic medicine and surgery into further 8 specialities as enumerated in the *Susruta Samhita.*

There are some divergence of opinion regarding the dates of these different historic persons and their works. This is mainly due to the confusion in the identity of the persons and also to the lack in the proper personal knowledge of the subjects. Castellani and also Garrison understand that Atreya taught medicine in Taxilla in 6th century B.C., and that the age of Charaka and Susruta was 2nd and 5th century A.D. respectively. All these dates have been greatly underestimated except, perhaps, the date of Charaka. In fact, Charaka appeared several thousand years after Agnivesa and Susruta.

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* Analysis of the contents of the *Nidanasthanam* (Section on Pathology) of the *Charaka Samhita* (8 chapters) :—

सर्व्वज्वरनिदानम	Pathology of Fevers	कुष्ठनिदानम्	Skin Diseases
रक्तपित्तनिदानम्	Hæmorrhage	शोषनिदानम्	Consumption
गुल्मनिदानम्	Tumours	उन्मादनिदानम्	Insanity
प्रमेहनिदानम्	Urinary Diseases	अपस्मारनिदानम्	Epilepsy and Hysteria

Analysis of the contents of the *Nidanasthanam* of the *Susruta Samhita* (16 chapters) :—

वातव्याधिनिदानम्	Pathology of the Diseases of the Nervous System	विद्रधिनिदानम्	Deep-seated Abscess
अर्शोनिदानम्	Hæmorrhoids	विसर्पनाड़ीस्तनरोगनिदानम्	Erysipelas, Sinus, Diseases of the Breast
अश्मरीनिदानम्	Calculus	ग्रन्थिअपचीअर्व्वुदगलगण्डनिदानम्	Tumours and Enlargement of Glands
भगन्दरनिदानम्	Fistula-in-ano	वृद्धिउपदंशश्लीपदनिदानम्	Scrotal Tumour, Venereal diseases, Elephantiasis
कुष्ठनिदानम्	Diseases of the Skin	क्षुद्ररोगनिदानम्	Minor Diseases
प्रमेहनिदानम्	Urinary Disorders	शूकदोषनिदानम्	Ulcer in Penis by Suka
उदरनिदानम्	Abdominal Tumour and Ascitis	भग्ननिदानम्	Fracture, Dislocation
मूढगर्भनिदानम्	Malformations of the Fœtus and Difficult Labour	मुखरोगनिदानम्	Diseases of the Mouth.

From a perusal of *Charaka Samhita* we find that every chapter of the work commences with words meaning that "the sage Atreya has thus taught and his pupil Agnivesa has written in his words." Indeed, *Charaka Samhita* is nothing but *Agnivesa Samhita* redacted by Charaka as mentioned by Charaka himself: **अग्निवेशकृते तन्त्रे चरक प्रतिसंस्कृते ।** Even at the present time we find several compilatory works written by **Vagbhat, Vrnda, Chakrapani, Dallana, Vijayarakshit, Srikantha** and others who flourished between the 6th to 13th century A.D., quoting passages from **Agnivesa Samhita** which are not to be found in *Charaka Samhita.* Sen rightly remarks that, at the time of Charaka, *Agnivesa Samhita* had become so much dis organised that it was required to be re-edited and redacted. This metamorphosed edition of *Agnivesa Samhita* is our present-day *Charaka Samhita.* This fact is further exemplified by the fate of the work of **Vela,** the second pupil of Atreya, namely the *Vela Samhita.* This work, which exists in the Tanjore State library and was quoted frequently by various previous workers, has suffered from such a great disorder that a redacted edition must fill up at least 30% of lost passages. This Tanjore manuscript has recently been printed (in modern Sanskrit script) by the Calcutta University.

Unfortunately the name Atreya appears in the Buddhistic literature as teaching medicine at Taxilla about the 6th century B.C., which has confounded the Western scholars. He was quite a different person and is known as **Vikshu Atreya** of the Buddhistic literature. Gana Nath Sen understands that he was the teacher of **Jibaka** who, in his turn, was the physician of the Buddha himself and of the Buddhistic monarch Bimbisara. Another point which must be remembered in this connection, is that, although Taxilla was the greatest seat of learning in the whole world at that time, and Vikshu Atreya was the most prominent teacher there, no mention of Taxilla (*Takshashila* in Sanskrit) ever exists in *Charaka Samhita,* although the names of the sages Atreya and Agnivesa exist at the beginning of every chapter.

Regarding Charaka, again, some confusion has taken place from a passage "**कठचरकाल्लुक्**" in the celebrated grammar *Panini* (6th century B.C.—Goldstücker), and hence it was assumed that Charaka was anterior to **Panini.** This is really so, but this Charaka is not the redactor of *Agnivesa Samhita.* He is the compiler of several hymns of *Yayurveda* (the 3rd Veda) which is anterior to Ayurveda and hence of a long period previous to Agnivesa or his teacher Atreya.—(Sen, *Ayurveda Samhita,* p. 17.)

The *Susruta Samhita* has also suffered from the same tale of disorganisation, redaction and interpolation. Thus **Nagarjuna,** the great Buddhistic teacher, was the redactor of *Susruta.* From the presence of several quotations in this edition from the redacted edition of *Charaka Samhita,* it is clear that Nagarjuna appeared after Charaka, and probably from this redacted edition of *Susruta* difficulty arose regarding the date of Susruta himself, and thus it has been wrongly fixed by the Western writers to be the 5th century A.D.

Then comes the third phase of the Indian School of Pathology, or rather, of Indian Medicine itself. This period commences with **Madhava Kara.** From his own writing we find that he was the son of Indu Kara. He was a Bengalee and wrote another book, *Ratnamala*, on the properties of drugs. His *Nidana*, along with *Charaka* and *Susruta*, was translated into Arabic by order of Harun-al-Rashid (786-802 A.D.). So the most recent date that could be assigned to him is the 7th century A.D. Wilson believes further that these translations were done either from the originals or, more probably, from translation made at a still earlier period into the language of Persia. This takes back the time of Madhava Kara to a further anterior period.

From Indian materials the age of Madhava Kara can be ascertained without difficulty. *Madhava Nidana* has been widely quoted by **Vrnda,** and both Madhava and Vrnda by **Chakrapani,** the author of *Chakradatta.* It is definitely known that Chakapani flourished in Bengal at the court of **Nayapala** who ruled at Gour at about 1060 A.D. From this date we take Vrnda to have flourished in the 9th century A.D. **Srimadhava,** the commentator of *Susruta,* and **Madhavacharyya,** the commentator of the Vedas, are quite different persons and must not be confounded with Madhava Kara, the writer of *Nidana.*

This third period of the history extended for a period of about 900 years (7th to the 16th century), *i.e.*, from **Madhava Kara.** to **Bhavamisra,** the writer of *Bhabaprakash,* with **Sorhala, Vrnda, Chakrapani, Sarngadhara, Dallana, Arunadatta, Vijayarakshit, Srikantha, Bangasena, Sivadasa** and others coming between them. The works of all these authors are now extant and form the basis of the studies of the present-day Ayurvedic physicians.

It is not the scope of this work to discuss how the last period, or the period of decline of the Indian School of Medicine commenced with the Mahomedan invasion of India, how the dastardly plunder and destruction of all culture by Mahmud Ghazni (11th century A.D.) followed by those of Muhammad Ghori (1191), Alauddin, Chengiz Khan (13th century), Tamerlane (14th century), the disturbances during the Pathan rule and the internal strife between the successors to the throne, intrigues, and suspension of all intellectual environments during the Mughal rule, with the single exception of the period of Akbar, have caused the downfall of Indian medicine. All these causes, aided by the patronage of the State help to the Unani and the Western Medical system during the Mahomedan and British rule respectively, have further accelerated its decline.

Analysis of the Contents of "Nidanam" by Madhava Kara

मङ्गलाचरणम्	Introductory Salutation	अर्शोनिदानम्	Hæmorrhoids
ज्वरनिदानम्	Pathology of Fever	अग्निमान्द्याजीर्णविसूचिकालसकविलम्बिकानिदानम्	Anorexia, Dyspepsia, Indigestion, Cholera and Tympanitis
अतीसारनिदानम्	Diarrhœa		
ग्रहणीनिदानम्	Chronic Diarrhœa		

क्रिमिनिदानम्	Worms
पाण्डुरोग कामला कुम्भकामला हलीमकनिदानम्	Anæmia, Jaundice, and Malignant Jaundice
रक्तपित्तनिदानम्	Hæmorrhage
राजयक्ष्मशोषक्षतक्षीणनिदानम्	Phthisis, Consumption, Ulcer
कासनिदानम्	Cough
हिक्काश्वासनिदानम्	Hiccough, Asthma
स्वरभेदनिदानम्	Hoarseness
अरोचकनिदानम्	Aversion to Food
छर्द्दिनिदानम्	Vomiting
तृष्णानिदानम्	Thirst
मूर्च्छाभ्रमसन्न्यासनिदानम्	Fainting, Giddiness, and Apoplexy
पानात्ययपरमदपानाजीर्णपानविभ्रम	Alcoholism
दाहनिदानम्	Burning
उन्मादभूतोन्माद	Insanity and Delusion
अपस्मारनिदानम्	Hysteria, Epilepsy
वातव्याधिनिदानम्	Convulsion and Nervous Diseases
वातरक्तनिदानम्	Leprosy
ऊरुस्तम्भनिदानम्	Paraplegia
आमवातनिदानम्	Rheumatism
शूलपरिणामशूलान्नद्रवशूलनिदानम्	Colic
उदावर्त्तानाहनिदानम्	Diseases caused by inhibition of natural functions, *viz.*, Anuria and stoppage of defæcation, Yawning, etc.),
गुल्मनिदानम्	Tumours
हृद्रोगनिदानम्	Diseases of Heart
मूत्रकृच्छ्रनिदानम्	Strangury
मूत्राघातनिदानम्	Retention of Urine
अश्मरीनिदानम्	Stone or Calculus
प्रमेहमधुमेहपिड़का	Diabetes and Carbuncles
मेदोनिदानम्	Obesity
उदरनिदानम्	Abdominal Diseases
शोथनिदानम्	Œdema
वृद्धिनिदानम्	Hernia, Hydrocele, Hæmatocele, etc.
गलगण्डगण्डमालापचीग्रन्थ्यर्ब्बुद-	Goitre, Scrofula, Enlargment of Glands
श्लीपदनिदानम्	Elephantiasis
विद्रधिनिदानम्	Cellulitis
व्रणशोथनिदानम्	Abscess
शारीरव्रणनिदानम्	Ulcer
सद्योव्रणनिदानम्	Wounds
भग्ननिदानम्	Fractures and Dislocations
नाड़ीव्रणनिदानम्	Sinus or Fistula
भगन्दरनिदानम्	Fistula-in-ano
उपदंशनिदानम्	Genital Ulcers
शूकदोषनिदानम्	Spermatorrhœa
कुष्ठनिदानम्	Diseases of the Skin
शीतपित्तोदर्द्दकोठ	Urticaria
अम्लपित्तनिदानम्	Acidity
विसर्पनिदानम्	Erysipelas
विस्फोटकनिदानम्	Boils
मसूरिकानिदानम्	Small Pox
क्षुद्ररोगनिदानम्	Minor Diseases
ओष्ठगतमुखरोग	Diseases of Lips
दन्तवेष्टगतमुखरोग	Gum
दन्तगतमुखरोग	Teeth
जिह्वागतमुखरोग	Tongue
तालुगतमुखरोग	Palate
कण्ठगतमुखरोग	Throat
सर्व्वसरमुखरोग	Face
कर्णस्रोतोगतरोग	Organs of Hearing
कर्णरोगनिदानम्	Ears
नासारोगनिदानम्	Nose
नेत्रसर्व्वगतरोग	Eyes
नेत्रकृष्णगतरोग	Iris
नेत्रदृष्टिगतरोग	Disturbance of Vision
नेत्रशुक्लगतरोग	Sclera
नेत्रसन्धिगतरोग	Joinings of the Eye
नेत्रवर्त्मगतरोग	Eye-lids

शिरोरोगनिदानम	Brain	मूढगर्भनिदानम्	Unnatural Labour
असृग्दरनिदानम्	Dysmenorrhœa and Menorrhagia	सूतिकारोगनिदानम्	Puerperal Diseases
		स्तन्यदुष्टिनिदानम्	Diseases of the Breast
योनिव्यापन्निदानम्	Vagina	बालरोगनिदानम्	Diseases of Children
कन्दनिदानम्	Prolapse of Uterus	विषरोगनिदानम्	Effects of Poisons

Castellani truly observes, "There is no doubt that the Indian doctors were well versed not merely in medicine and surgery, but also in the prevention of disease and operative midwifery. They apparently knew diabetes mellitus, dysentery, phthisis, syphilis and diseases due to worms, etc. In diagnosis they paid great attention to the examination of the pulse, the temperature of the body, the colour of the skin, the fæces, eye, voice, and the respiratory sounds. They possessed a remarkable symptomatology, and, as regards treatment, divided diseases into *incurable* which they refused to treat, and *curable* which they treated according to a copious materia medica Among the various remedies must be mentioned the fact that inoculation of small pox was practised at the beginning of the warm season. Dietetics and toxicology were also well-known. Hospitals were founded by Buddhist princes in India and Ceylon. In fact, there is an account of a hospital being founded in Anuradhapura, the ancient capital of Ceylon, as early as the fifth century B.C., and later many more were established, as well as a sort of medical or sanitary department, having one medical officer to every ten villages, together with a definite sanitary organisation, and with institutions for the reception of cripples, deformed, and poor persons."—(Castellani and Chalmers, *Tropical Medicine*.)

Post-mortem examination was also practised by the ancient Hindus Mukhopadhyay observes, "But, though we do not find reference to post-mortem examination in the law-books and medical literature of the Hindus, we find it mentioned in the *Arthasastra* of Kautilya as *Asumrtakapariksa* (Examination of bodies of men who have recently died). Report of such an examination was necessary in higher courts of law (*Kantakasodhana* Courts) during the reign of Chandragupta. Bodies of men who committed suicide by hanging, drowning, poisoning, etc., were kept in an 'Examination Room' which was set apart for the purpose. Such examination rooms, corresponding to our morgues, were established in the different provinces of the Empire. Here all accidental cases, homicidal or suicidal, were kept for examination and reported, as to the cause of death, to higher authorities who dealt with the cases accordingly. To prevent decomposition, dead bodies were kept immersed in oil or oleagenous preparation"—*Asumrtakapariksha* in the *Arthasastra*, pp. 215-217.—(*History of Indian Medicine*.)

The Antiquity of Indian and Greek Medicines

Thanks to the modern researches (though still on a modest scale) that are being conducted into the etiology and theory of Ayurveda, a new interest in this indigenous system of medicine has been aroused not only among Indians as such but Western scholars including medical men. Ignorance, however, has not been completely replaced by light, as is shown by the following statement of Sir Asutosh Mookherjee:—

> "It needs no elaborate argument to establish that this 'inexhaustible fount' (of the Sanskrit literature) requires for its complete exploration an army of scholars; and till this has been achieved, summaries of the history of Indian medicine must continue to be incomplete and fragmentary. We cannot consequently express surprise when we find that Neuberger, in his great work, devotes only 18 pages to the subject; while Prof. Buck of Columbia, in his work on the growth of medicines from the earliest times to the 18th century, contents himself with a few paragraphs which occupy 8 pages only. In these circumstances, it is no wonder that the topic is not even mentioned in the brilliant lectures on 'the evolution of medicine' delivered by Sir William Osler at the Yale University in 1913."

The student of medicine, however keen or earnest in his introspection, has his vision to-day generally bounded by the age of **Hippocrates**, popularly called the "Father of Medicine." Here in India is a system of medicine dating back to a time no man can yet positively tell, which has obviously stood many shocks and tribulations, and though apparently decadent to-day, is the only method of treatment in vogue for the great bulk of India's 350 millions.*

Between the age of Hippocrates and the age that witnessed the evolution of Ayurveda there apparently yawns a deep gulf, and it is but natural that the keen student of history of medicine will be curious to bridge this. To this end have laboured a distinguished band of European and Indian scholars like Royle, Wise, Hœrnle, Muthu, Bhagabat Singhji, Mukerjee and many others. Considerable light has also been shed on the subject by the publication of the ancient literature of foreign countries like Greece and Arabia relevant to this subject, which not only proves the hoary antiquity of the Hindu system of medicine but helps to re-discover the link between Hippocrates and the age beyond. The first string of questions that will naturally arise in this connection will be:—Is the origin of Ayurveda anterior to the age of Hippocrates, or *vice versa?* Was there any rational connection between Ayurveda and the ancient system of Greek Medicine? If so, did Ayurveda borrow its essential features from the Greek system, or *vice versa?*

The second question in favour of which evidence is multiplying day by day, has lent an additional interest to the first and the third. From evidence, both external and internal, it is now a matter of more than mere idle speculation that there was an essential affinity between Ayurveda and the healing art of the old Greeks, which latter is said to have given birth to modern medicine.

Asoka (*c.* 274-237 B.C.) was a great monarch in whose court there was a congregation of international physicians, and it is said that when Alexander the Great came to India (327 B.C.), the Greek physicians who accompanied him made an earnest study of Hindu medicine on the spot. Nay more—when the invader finally left the Indian soil he carried with him to his court a number of Hindu physicians. At a later period, the Arab manuscripts, which were composed at the time of Harun-al-Rashid and which have since become public property. disclose that, during his reign, the great monarch called to his court a Medical Congress of the representatives of the Hindu, Greek, Arabic and Egyptian systems of medicine and had, amongst others, the principal tenets of the Hindu medical practice translated into Arabic. The congress was not exactly like a Medical Congress of modern times, the session of which is "short and sweet" It was convened for a set purpose—not merely for an airy interchange of ideas but with a view to a synthetic amalgamation. However that might be, it cannot but be supposed that the Hindu and Greek system of medicine were mutually influenced—and perhaps deeply—as a result of this congress.

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*According to a statement of Sir Surendra Nath Banerjea, made when Minister of Public Health in Bengal, the proportion of Western medical aid carried to the masses is 1 to 45,000. Kaviraj Gananath Sen contends that at least 90 per cent. of the rest are treated by the Ayurvedic method.

Much of these statements is perhaps speculative, but there is enough external evidence of sufficient historical value to show that occasionally there was intimate contact established between the old Greek and the Hindu system of medicine. The internal evidence that is now being increasingly supplied by some of the latest developments of Western therapeutics, is strengthening the theory of a fundamental affinity between Ayurveda and the old Greek medicine. Garrison says:—

> "In pathology, the plastic signification of the number four (the sacred numeral of **Pythagoras)** was combined, in the teaching of Plato and Aristotle, with the doctrine of the four elements as follows: Corresponding with the elements of earth, air, fire and water were the qualities dry, cold, hot and moist according to the scheme—
>
> hot+dry = fire   cold+dry = earth
> hot+moist = air,   cold+moist = water.
>
> Long before **Aristotle,** probably before Hippocrates, it was held that, corresponding to the four elements fire, air, water and earth and the four qualities hot, cold, moist and dry, are the four humours of the body, *viz.*, blood, phlegm, yellow bile and the black bile. These three sets of elements, qualities and humours could then be brought, by permutation and combination, into a complex system of arrangements based upon the following scheme—
>
> hot+dry = yellow bile   cold+moist = phlegm
> hot+moist = blood   cold+dry = black bile
>
> the different combinations giving the qualitative aspects of disease, and, by the same token, of the physiologic action of the drugs. The whole arrangement made up the 'humoral pathology,' which regarded health and disease as the proper adjustment or imbalance respectively of the different components mentioned."

Further, we can take for instance another exposition of the same as noted by Garrison:—

"The doctrine of Hippocrates, as brought forward in various parts of his work, is that the body itself is supposed to consist of four elements, combined in different proportions in different individuals. The combination of the four elements into four states or qualities with which they were affected—of hot, cold, moist and dry—gave rise to the four fluids or humours of the body, blood, phlegm, yellow and black bile, which originally tended to produce the four temperaments, and which, in their turn, contributed to the excess or defect of each of the humours."

Hence arose the pathological doctrines which, under the denomination of humoral pathology, became the prevailing opinion of all sects and of all theorists until the commencement of the 18th century.

More remarkable than this is the almost self-evident proof of an affinity between the old Greek humoral pathology (blood, phlegm, black and yellow bile) and the Tridosha theory of Ayurveda (vayu, pitta and kapha) which is said to be the bed-rock of Ayurvedic therapeutics. Meantime, the question becomes irresistible in the face of the remarkable affinity between the Hippocratic doctrine of humoral pathology and the Tridosha theory of the Hindus as to whether the first was the outcome of the second or *vice versa*. To assert that the affinity is merely accidental is to fly in the face of facts and strong probabilities. Western scholars, on the whole, have been lately impressed by this affinity and other marks of similarity, but, through their ignorance of Sanskrit and through their hazy conception of India's ancient culture, they are disposed to yield the palm to the old Greeks. But it is evident that the balance of probabilities is in favour of just the other way. Hippocrates lived between 460-370 B.C., and he it was who propounded the Greek humoral theory. There is, therefore, a historical definiteness about the origin of the Greek theory. On the other hand, no definite or even approximate date can be gathered as to the origin of the Hindu medical system. The date may well be pre-historic, and Ayurveda, as the name suggests, may be as old as the Vedas themselves, which European scholars admit to be the oldest books in the world. In fact, there are significant passages in the Rg Veda which unmistakably point to a sufficiently consolidated system of medical treatment to be in vogue at the time.

Opinion is overwhelmingly in favour of this affinity being borrowed from India by the Greeks, either directly by their visits to India or through the Egyptians. Royle describes how the ancient Greeks acquired their knowledge from the East. The Egyptians, from whom the Greeks directly obtained their source and " who existed as a powerful and civilized nation under the Sesostridæ, from B.C. 1600 to B.C. 800 and were ruled over by the Hukos from 1800 B.C. to the former period, seem in very early ages to have been acquainted with the products of India."—(Royle, *An Essay on the Antiquity of Hindu Medicine*, 1837.)

At a much later period "India was visited by Phthagoras, Anaxardes, Pyrrho, and others who afterwards became eminent philosophers in Greece."—(*Brucker's Philosophy* by Enfield.)

Royle further mentions:—

> "Hippocrates (450 B.C.,) is stated to have studied medicine under Herodicus and is classed among the followers of Pythagoras and, like this philosopher, is described as having spent a considerable portion of his life in travelling in foreign countries."

Further, the internal evidence of the published works of Hippocrates shows clearly the way of origination of this affinity. It has been remarked:—

> "That Hippocrates derived assistance from the Hindu, is evident from the number of Indian plants imported from there to Greece, mentioned in his materia medica and used for their well-known properties by the Greeks"—(*Ayurveda*, p. 15)

On the other hand, opinion is overwhelmingly against this affinity being borrowed by Indians from Greece. Royle mentions:—

> "The description of some diseases which seem to have been first known in India, as well as the internal administration of metals they could not have borrowed from the Greeks. Many of the Indian medicines may be found described in the works of the Greeks, but we see no trace of European medicines in those of the Hindoos." Further, "Dietz proved that the late Greek physicians were acquainted with the medical works of the Hindoos, and availed themselves of their medicaments."

Royle, Wise and others say that "Pythagoras, Empedocles, Democritus, Plato, Thales, Creates, Endoxus and others travelled to the East." "On the other hand, we never hear of Indians visiting other lands for acquiring the wisdom of those countries. Neither do we find of any authentic reference to *Yavanacharyas* (foreigners) from whom they have taken their knowledge of medicine or philosophy in any of their books. They have done so in astrology which they distinctly mentioned."—(Roy.)

Leaving aside the earliest or the Vedic period of Indian medicine, when we come to the much later period, or the period of *Samhitas* or original works such as *Agnivesa Samhita* (later redacted by Charaka and known up to this day as the *Charaka Samhita*) and *Susruta Samhita*, the antiquity of this period still seems far to outdistance that of the age of Hippocrates:—

> "Hessler, in his Latin translation of *Susruta Samhita*, assigned the appearance of this work to a remote period of Indian history, the beginning of which is lost in the immensity of time and the end of which is known to be about 1000 B.C."—(Mukhopadhyay, *History of Indian Medicine*.)

Hœrnle and others are also of the same opinion. It is evident from Charaka, Susruta and other ancient literature, which gave practically the final shape to the system of Ayurveda as practised at this day, that the origin of Ayurveda is still within the pre-Budhistic period. It can, therefore, be definitely stated that the fundamentals of Ayurveda—and specially the theory of Tridosha—were not borrowed from the Greeks or, as a matter of fact, from any other nation.

Like the schools of Indian Philosophy, Indian Mathematics (*Ganita*), Indian Geometry (*Bhoomity*), Indian Trigonometry (*Triconomity*) and many other intellectual sciences, the credit for whose initiative is sometimes unjustly, apportioned, the fundamentals of Ayurveda are undoubtedly indigenous to the Indian soil. Taylor's remarks on this point are significant. He (Taylor—*Jl. Roy. Asiatic Soc.*, Vol II, 1834, cited by Pococke, Appendix No. XVI) says:—

> "It was an astounding discovery, that Hindoosthan . . . . possessed . . . . a philosophy, compared with which, in points of age, the lessons of Pythagoras are but of yesterday; and in point of daring speculation, Plato's boldest efforts were tame and commonplace. A poetry more purely intellectual than any of those of which we had before any conception; and systems of science whose antiquity baffled all powers of astronomical calculations."

Wise (*Commentary on the Hindu System of Medicine*, 1845) rightly remarks:—

> "Nor do the Hindoos appear to have derived their knowledge from any of their neighbours. The Greeks were the only people from whom they could have borrowed their information; but their having borrowed their literature seems to stand on an exceedingly slender basis."

It is thus a strain upon ordinary belief that the ancient Hindus, who had outstripped other nations including the old Greeks in so many useful branches of human thought, would wait till well after the 4th century B.C., to learn the Hippocratic Theory of Pathology and to evolve on this basis their system of medicine.

I quote the following from Pococke (*India in Greece*, 1852):—

> "The Greek language is a derivation from the Sanskrit; therefore Sanskrit-speaking people, *i.e.*, Indians, must have dwelt in Greece, and this dwelling must have preceded the settlement of those tribes which helped to produce the corruption of the old language." "Not only her language, but her philosophy, her religion, her rivers, her mountains, and her tribes; her subtle turn of intellect, her political institutions, and above all the mysteries of that noble land,—irresistibly prove her colonisation from India:"

This is further corroborated by other writers. Thus, the learned Dr. Prichard (*Physical History of Man*, Vol. 1, p. 502) says:—

> "The affinity between the Greek language, and the old Parsee,. and Sanskrit, is certain and essential. The use of cognate idioms proves the nations who used them to have descended from one stock. That the religion of the Greeks emanated from an eastern source no one will deny. We must, therefore, suppose the religion, as well as the language of Greece to have been derived, in great part, immediately from the East."

That the Greek language is the direct outcome of the Sanskrit, is shown by Royle who records the observation of Mr. Princep, Secretary of the Asiatic Society:—

> "On the very great similarity between the old Sanskrit and the Greek characters, more palpable the further we retire into antiquity, the older the monuments we have to decipher, so that we might almost advance, that the oldest Greek was nothing more than Sanskrit turned topsy-turvy." "The

> great language has besides been now indisputably proved to be but a branch of the Sanskrit stem."—(Royle, p. 196).

Thus, it is clear that earlier Indian settlers in Greece carried with them Indian Philosophy and Medicine through Sanskrit. There might have been further introduction of the same, indirectly through the Arabs and the Egyptians, into Greece long before the age of Hippocrates.

Conversely, therefore, the originality of the Hippocratic theory suffers as that the Ayurvedic theory is established. Lietard (quoted by Muthu) justly observes:—

> "As the theory of Humoral Pathology was shared by both the nations, if it can be proved that the doctrine was broached in India anterior to the time of Hippocrates, not only would the originality of the Hindus be established but that of the Greeks be compromised thereby."

In the review on the *Commentary on the Hindu System of Medicine* by Wise, the *Lancet* (Sept. 12, 1846) rightly remarks:—

> "None but those who are acquainted with the languages of the East, can form any idea of the surprising knowledge of this ancient people, anterior to that of the Greeks or Egyptians.
>
> "Their geometry, arithmetic, and astronomy surpassed that of Ptolemy; their music, that of Pythagoras; their mechanics that of Archimedes; their theology, that of Plato; and their logic that of Aristotle."

It is, therefore, more than probable that ancient Greece directly or indirectly owed her Medicine—especially the famous Humoral Pathology—to the ancient Hindus. It may be the Arabs first learned it from the Indians, who communicated it to the Egyptians, from whom, again, the Greeks finally derived the Hindu medical knowledge. More probable is the theory of direct communication. Recent researches into the realm of ancient Indian history have exploded many old dogmas and shibboleths about the immobility of the early Hindus and established new "values" about their national character, the knowledge of which, however, has not yet widely diffused among European scholars. It has been reasonably proved that the ancient Hindus were a highly maritime, enterprising and colonial race. As shown by the latest excavations in Java and Bali island, they actually established colonies in those places in some dim period of history. Pococke remarks:—

> "That the people of the country of the Indus ranked as navigators, in the most venerable antiquity, is prefectly clear from the ancient *Institutes of Menu,* where merchants who traffic beyond the sea, and bring present to the king are expressedly mentioned. These *Institutes of Menu* running up to the vast antiquity of B.C. 1400, give an idea of the early commercial energies of India."

Colebrooke, the great Indologist, made a remark which is very significant. He says that there was a greater degree of similarity to be found between the early rather than the later Greeks, among whom Pythagoras, Hippocrates, Plato, Aristotle and many others can be strictly included. The theory of Indian colonisation in Greece, the Indian colonists carrying with them the seeds of Hindu culture including Hindu Medicine and sowing them on the virile Greek soil, though seemingly extravagant is perfectly tenable in the light of recent historical researches. According to Pococke:—

> "The primitive history of Greece is the primitive history of India. It is the history of much of India, in its language, in its religion, in its sects, in its princes and bravest clans. Nay more, I shall exhibit dynasties disappearing from Western India to appear again in Greece; clans whose martial fame is still recorded in the faithful chronicles of North-Western India, as the gallant bands who fought on the planes of Troy; and in fact, the whole of Greece, from the era of the supposed God-ships of Poseidon and Zeus, down to the close of the Trojan war, as being Indian in language, sentiment and religion, and in the arts of peace and war."

The Indo-Greek colonial theory can well be stretched on similar grounds so as to make it apply to the cases of Egypt, Persia, Babylonia, etc.—countries which early saw the germination of civilization. Indeed, there are so many points of similarity in the early phases of civilisations attributed to these ancient lands that there must have been a common ground of contact and an orginal source for all of them. The probability is the strongest that it was the Hindus who were the inspirers of these civilisations and the banks of the Indus were the world's clearing-house for the dissemination of ideas as well as of races. In short, the early Hindus were the world's first teachers and the world's first colonial power.

WESTERN SCHOOL

Hippocrates (460-370 B.C.), the greatest of all physicians and universally called the *Father of Medicine*, advocated that all diseases are result of disorders of the fluids of the body—the *Hippocratic Doctrine of Humoral Pathology*. This doctrine ruled the medical world without any rival, until **Asclepiades** (124 B. C.), opposed this and formulated the doctrine that diseases are caused by contracted or relaxed conditions of the solid particles of the body. Thus *Solidism* came in opposition to *Humouralism*. From this doctrine came the theories of *sthenic* or *asthenic* and *tonic* or *atonic* conditions of the body as also the theory of *irritation* or *stimulus*, etc.

Aristotle (384-322 B.C.), the most distinguished pupil of Plato and instructor of Alexander the Great, many be regarded as the *founder of natural history*. He observed, from day to day, the development of the chick within the egg, and divided the structure of the animal frame into organs and tissues. Dissection of the human body was legalised in 300 B.C., and that of the bodies of condemned criminals only. The earliest pioneer in the study of anatomy was **Galen** (130-200), the *founder of experimental physiology*, who was the greatest of the Greek physicians next to Hippocrates. In his voluminous writings Galen contributed no less than six books on pathology. He advocated the doctrine of *Pneuma* or a spirit pervading throughout the whole of our body, and thus formulated a system of pathology which combines the **Humoral** theory of Hippocrates, the **Four-Element** theory of Pythagoras and the **Pneuma** of himself.

Galen has given us "the four classic symptoms of inflammation, differentiated pneumonia from pleurisy, was the first to mention aneurism, described the different forms of phthisis, mentioning its infectious nature and proposing a full milk diet and climato-therapy for the disease; he understood the diathetic relation between calculus and gout, and his prescriptions indicate a most intelligent use of opium, hyoscyamus, hellebore and colocynth, hartshorn, turpentine, alcohol (wine), sugar diet (honey), grape juice, barley water and cold compresses. He introduced the doctrine of the four temperaments and set the pace for a fantastic pulse-lore which vas still in vogue in the 18th century.

"He was the first to describe the cranial nerves and the sympathetic system, made the first experimental sections of the spinal cord, producing hemiplegia: produced aphonia by cutting recurrent laryngeal; and gave the first valid explanation of the mechanism of respiration. He showed that the arteries contain blood and demonstrated the motor power of the heart by showing that the blood pulsates between the heart and a ligated artery, but not beyond it"—(Garrison's *History of Medicine*).

The true study of anatomy began with **Vesalius** (1514-1564) and his contemporaries **Eustachius** and **Fallopius** (1523-1563).

Vesalius's observations were supplemented by **Harvey** (1578-1667) who introduced experimental methods for the study of the function of the structures. His experiments were mainly on frogs and dogs, and, to a certain extent, on human beings also. He was the first to demonstrate that the whole quantity of blood in the animal body moves in a circuit, and that the propelling force is supplied by the beating of the heart.

Thus, the observations of Vesalius and Harvey were complementary, in the sense that those of the former related to the structure, and of the latter, to the function of that structure—the two aspects of biology of the living organism.

Simple lenses or magnifiers which had been known to the people since the time of the ancient Greeks and Romans, underwent great developments, but the effect of combination of lenses was never thought of until **Janssen** (1580-1616), a Dutch spectacle maker, and his son accidently discovered that two lenses, when put together, give a higher magnification than either of them does singly, a fact which gave birth, in *circa* 1590, to a microscope consisting of a series of lenses in a copper tube 6 feet long and one inch in diameter, **Gallileo** (1564-1642) studied the laws of refraction of a system of lenses and thus constructed a compound microscope, about the year 1600, based on scientific principle. The greatest improvement was made by **Leeuwenhoek** (1632-1723), who manufactured a large number of microscopes which gave the highest magnification then possible, namely, about 160 diameters.

By the beginning of the 19th century much improvement was done to the microscope as regards magnification, but the greatest difficulty was with chromatic abberation, as the lenses used were merely spherical and of short foci. To obviate this difficulty, **Modena**, in 1812, and subsequently, other physicists tried a combination of concave and convex lenses of different curvatures, until, in 1837, excellent microscopes with achromatic lenses of higher magnification and clearer definition were available. The use of the water-immersion lens was discovered by **Amnici**, and subsequently, improved by **Hertnack** in 1855. **Wenham** (1870) substituted cedar-wood oil instead of water, and, in 1878, **Abbe** manufactured the oil-immersion lenses. Abbe also constructed an apochromatic objective in 1880, and the substage condenser which greatly improved the construction of the microscope.

The history of the development of the miscroscope presents us with two of its devotees in the persons of **Malpighi** (1628-1694) and **Leeuwenhoek.**

Malpighi made numerous discoveries having practical bearing on anatomical and physiological advances as related to medicine. In 1661 he demonstrated the presence of air cells in the lungs and the mechanism how the air was brought to the blood in the lungs, always however, being separated by means of a membrane. He also observed the passage of blood through the capillaries of the lung of the frog, and also observed the blood corpuscles. He demonstrated the pigmented layer lying intermediate between the true and the scarf skin, which is known after his name. His observations on other structures, *e.g.*, the nature of the papillæ on the tongue, Malpighian corpuscles in the kidney and spleen, have made him immortal in the annals of medicine. But more credit is due to him for his studies on comparative anatomy, botany and zoology.

Leeuwenhoek never crossed the threshold of any university. He left school at the age of 16, and joined a tailoring firm where he worked as book-keeper and cashier. A few years later, he gave up service and devoted himself to the study of natural history. He greatly interested himself in the microscope which had been invented recently, and though he had never any systematic training in science, he began to subject, under microscopic observation, anything that came in his way in the mineral, vegetable and the animal kingdom. In spite of his imperfect training, he grew to be a master observer, and, in the course of 50 years (1673-1723) more than 400 publications passed through his hands.

His startling discovery was about the circulation of the blood through the capillaries which connect the artery with the vein. This actually demonstrated the fact which Harvey had supposed, but had never actually discovered, during his experiments, blood flowing in circuit through the heart. Malpighi also had observed the capillary in the lungs of the frog, as well as the corpuscles, but Leeuwenhoek went further, and his observations were in detail. He studied the heart muscle, structure of the lens, spermatozoa, etc. He examined water and discovered in 1675 the minute animalcule (now called the *protozoa*) and in 1687 the *bacteria*, and thus has justly been denominated the *father of Protozoology and Bacteriology*. Both Malpighi and Leeuwenhoek observed honeycomb-like cavities in plant tissues, what they called "cells." This was the foundation of the "cell theory."

In spite of the incoherency of his work, Leeuwenhoek won the greatest admiration and appreciation from all quarters. In 1680 the Royal Society of London, which received no less than 375 papers from him for publication, admitted him as a fellow; the French Academy of sciences elected him a member in 1697, and in 1698, Peter the Great honoured him with a visit for the inspection of his microscopes and for making observations through them.

The discovery of minute animalcules by Leeuwenhoek, which are called protozoa since 1845, stimulated various workers, notably Ehrenberg, Stein, Schaudinn and others, who enriched the science of protozoology.

Cuvier made a profound study in comparative anatomy and studied systematically the structures of the organs and the tissues composing them. But **Bichat** (1771-1801) went a step further, and studied the elements which formed the tissues, and thus laid the foundation of histology (1800). Bichat died at the early age of 31.

The discovery of the nucleus in plant cells by **Brown** in 1831, and protoplasm by **Dujardin** (1801-1882) in 1835, laid the foundation of the "cell theory," which was thoroughly investigated and formulated by the brilliant researches of numerous workers, specially **Johannes Mueller** (1801-1858), **Schleiden** (1804-1881), **Schwann** (1810-1882), and **Schultze** (1825-1874). The "cell theory," as formulated by these workers, include the following conceptions:—

"(*a*) the cell as a unit of structure;
"(*b*) the cell as a unit of physiological activity;
"(*c*) the cell as embracing all hereditary qualities within its substance;
"(*d*) the cell in the historical development of the organism."—*Locy.*

Bichat thus opened a new line—*histology*—which was subsequently followed by a host of illustrious men specially **Schwann**, **Schultze**, **Henle** (1825-1874), **Virchow** (1821-1903), **Ramon y Cajal** (born 1850), and many others.

Not only the structure but also the functions of the cells, tissues, and organs have been extensively studied. Since the time of Harvey, who laid down the foundation of physiology, it has developed side by side with anatomy.

Charles Bell (1774-1842) showed that the nerve fibres of the anterior roots of the spinal cord belong to the motor type, whereas those of the posterior roots are of the sensory type.

Johannes Mueller (1801-1858), the greatest physiologist of modern times, with his distinguished pupils **Du Bois-Reymond** (1818-1896) and **Helmholtz**, and also **Claude Bernard** (1813-1878) have advanced physiology to an unparallelled extent.

Thus, normal anatomy and physiology were thoroughly established by the middle of the nineteenth century: people had already commenced to study the pathological anatomy and pathological physiology of different diseased conditions.

The following, among others, may be called the **Makers of Pathology** (including Parasitology and Immunology).

MORGAGNI

Morgagni, *the father of Pathology,* an Italian, was born on 25th February, 1682. After his preliminary education at his native village, he went to the neighbouring university town of Bologna for higher studies.

Just as in modern times Germany is regarded as the holy temple for the study of medical science, so the university of Bologna remained a sacred shrine for medical students during several centuries. Bologna is proud of producing master minds like **Vesalius** (father of anatomy)—who was born in Belgium, though Bologna and Pisa won the distinction of being the cradle of all his inventions and discoveries—**Fallopius** (abdominal anatomy), **Eustachius** (cranial anatomy), **Spigelius** (anatomy of the liver), **Malpighi** (microscopical anatomy), **Harvey** (discoverer of the circulation of blood), **Valsalva** and many others. Harvey, the Englishman, got the idea which led to his monumental discovery while working at Bologna.

Here Morgagni found his teacher in Valsalva, and before he was 21 years old, he was so much appreciated that, occasionally, he had to give lectures during the absence of his teacher Valsalva. His lectures as professor at Padua attracted people from all parts of the world, and here he founded the new sciences of pathological anatomy. He investigated the post-mortem findings in order to correlate the pathological findings and the clinical symptoms and the probable etiology of the morbid conditions. Systematic examination of all organs was his practice, whereas his predecessors remained satisfied with the examination of the organ supposed to be the seat of the lesion. Morgagni was the first person to investigate the following relationships:—

(*a*) Certain morbid conditions of organs occurring in connection with certain symptoms

(*b*) The same morbid condition ever occurring without those symptoms.

(*c*) Other morbid conditions causing same symptoms.

These amazing observations of facts have eminently entitled him to occupy the position of father of pathology, and his book entitled *The Seats and Causes of Diseases* is the classical work on the subject and form, the foundation of pathology. His works on sympathetic nervous system, aneurism, tuberculosis, cancer, etc., were also extraordinary.

He died at the age of 87 years, and long after his death the Italians commemorated his memory by starting a first-class medical journal called *Il Morgagni* published at Milan, which is still one of the best journals of the world.

VIRCHOW

Rudolf Virchow, *the father of Cellular Pathology,** was born on 13th October, 1821, at Schivalbein, a little town of Germany He was the son of a small merchant. Before he was seven, he could read and write, which he had learnt at home while playing with his father. By the age of nine he was well equipped in history, religion and Latin. Before the age of 13 he joined the high school (gymnasium), and at the age of 18 entered the Friedrich Wilhelm Institute in Berlin, where he received State aid for the purpose of training as surgeon for the Prussian army. He graduated at the Berlin University at the age of 22.

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*People all over the world used to think (and the advocates of the Ayurvedic system of pathology still think) of the pathological processes in terms of humoral pathology (the *Bayu, Pitta* and *Kapha* of the Ayurvedic system, and biliousness, catarrh, phlegmatic, etc., of old writers). Virchow commenced first to think of them and formulate the whole of pathology in terms of cellular changes, which has completely revolutionized modern medicine.

Virchow was the able pupil of his illustrious teacher **Johannes Mueller** (1801-58), *the founder of scientific medicine in Germany*. As he had at his side the illustrious pupils **Schwann, Henle, Virchow** and **Koelliker** as histologists, and **Du Bois Reymond** and **Helmholtz** as physiologists, one finds in Mueller an authority in biology, physiology, and comparative morphology.

The two astounding discoveries of Schleiden and Schwann during Virchow's student career made a great impression on him and this laid the foundation of his magnificent edifice of cellular pathology.

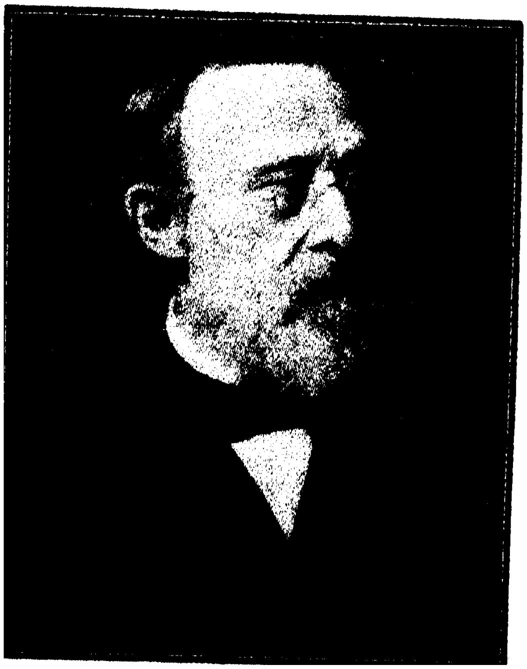


Fig. 1. Rudolf Virchow.

The epoch-making discovery of Schleiden was that plants are composed of cells which are independent elements of the organism. Theodore Schwann was the first to demonstrate and teach that all living tissues, whether animal or plant are composed of number of minute elements, as also to point out their significance, that they, under all circumstances, are biologically equivalent. His cell theory has now become the cell doctrine and Schwann has become the *Father of Modern Biology*. Virchow was in close relation with both Schwann and Schleiden, and as the cell theory was at once taken up by his teachers, Virchow learned very early to think in cellular terms. In 1846 he was appointed as prosector at the Charite Hospital in Berlin and his first lecture was on *Inflammation of Muscles*.

In Germany, Pathology has grown round the post-mortem table. **Rokitanski**, the leading pathologist of Virchow's time, had made 70,000 autopsies, and Virchow too made his studies entirely at the autopsy and without any clinical material. His next special studies were on leukæmia, embolism and thrombosis, septic infections, inflammation of blood vessels, etc.

In 1847, there occurred a severe epidemic of typhus in Silesia, and the health department could not do anything to control it. At last it became so serious that it attracted the attention of the Legislature, and a medical Commission, with Virchow as its medical adviser, was deputed to investigate the condition. He found that the social condition of the province was awful, and was so shocked that he sent a report to the Berlin Medical Society emphasing the fact that the bad social condition of the place was responsible for the origin and continuance of the epidemic, and he added that it was a disgrace to civilization in general and to the Prussian Government in particular. These strong remarks were too much for the Government to swallow and Virchow was dismissed from service, but was afterwards reappointed through the influence of his friends, though with an extremely limited scope. The University of Wurzburg at once invited him to the chair of an ordinary professor in the medical faculty.

Thus in 1848 Virchow, a pure scientist, was dragged into politics on his way to national fame. The last event taught Virchow a lesson that rash expressions only cause unnecessary provocation and serve no useful purpose. With this aim in view he started a journal called *The Medical Reform* which continued during the period he was at Wurzburg. At Wurzburg, he contributed a series of very valuable papers on bone diseases, tubal pregnancy, cretinism, echinococcus, amyloid degeneration, etc., and edited the handbook of special pathology and therapy.

These monumental works opened the eyes of the Prussian Ministry of Education, and, ultimately, the authorities decided to ask him to come back to Berlin. Virchow was thus reinstated as professor of pathology at Charite, where he spent the rest of his life, nearly 50 years. Soon after his return to Berlin (1858), he delivered a course of 20 lectures which appeared in the form of a book bearing the title *Cellular Pathology as based upon Physiological and Pathological Histology* This was the greatest medical book published in the nineteenth century. Shortly after its publication, it was translated into all the modern languages.

Four years after his graduation (1847) he founded the *Archiv* for pathological anatomy, now the best journal on pathology in the world, known as *Virchow's Archiv.*

The pathological institute of Berlin became the centre of teaching of pathology, and, among the assistants of Virchow, one finds the names of **von Recklinghausen, Klebs, Conheim, Orth, Roth, Grawitz** and many others, who themselves were founders of schools of pathology. Since Virchow's time the pathological institute at Charite has been regarded as a place of pilgrimage for the study of pathology.

Virchow not only made epoch-making observations on Addison's disease, small-pox, tuberculosis, syphilis, leprosy, pellagra, typhus, Basedow's disease, tumours, leucocytosis, leukæmia, spina bifida, arteritis deformans, and specially on thrombosis and embolism, but he was also one of the biggest anthropologists of the time. He had a fine collection of skulls illustrating the history of mankind for 6,000 years.

For over 40 years he served as a member of the Lower House of the Prussian Legislature. He was also the Alderman and took a keen interest in the health of the city. He was often compelled by his duties in the Legislature to remain in the House until midnight or even later; but no matter how late the hour, he would never go to bed until he looked over the reports of the diseases during the preceding 24 hours among the workers of the sewage firms.

He used to sleep for less than 5 hours but lived for more than 80 years: his death was the result of an accident causing an intracapsular fracture of the hip.

PASTEUR

Louis Pasteur, *the father of preventive medicine,* was born on 27th December, 1822. At the age of 25, Pasteur made himself famous in chemistry and molecular physics. In 1857, he was appointed the director of scientific studies in the Ecole Normale, and in the same year, took the scientific world by surprise by the publication of his great discovery that fermentation is the result of growth of an organic life, and, not due to any action of inorganic matters. This was the starting point whence Pasteur, a chemist, turned to a different subject, and devoted his energy more to microbes and diseases, and less to chemistry—crystallography being his favourite subject.

Fig. 2. Louis Pasteur.

Pasteur had to face severe opposition at every step of his researches, but came out successfully through every one of them.

His discoveries on the method of prevention of infectious diseases started from that of silk-worm plague, and then, of fowl cholera, anthrax, hydrophobia, etc. These observations have created a new line of thought amongst the scientists all over the world.

It was a known fact that a solution of sugar, if kept exposed to air, will show certain changes with production of gas and formation of alcohol. Similarly, milk, butter, or meat will also show changes, if kept exposed for some time. Pasteur was the first to show that these chemical changes are due to the presence of living agents called *ferments.*

Since the time of Aristotle (325 B.C.), and even earlier, the popular belief was that living things were generated spontaneously from non-living materials (the doctrine of *abiogenesis* or *spontaneous generation*). It was believed that frogs originate from the mud of ponds and streams through sun's rays, insects from the dew, and that mice breed by putrefaction, and so on.

Redi, the Italian, made some experiments in 1668, and, suggested, that the supposed products of life from non-living matter were always due to introduction of living germs from outside. Several years after this, Leeuwenhock discovered the protozoa, and subsequently, the bacteria. The theory of spontaneous generation was revived and it was held that bacteria are the first stage of life originating from the inorganic material through natural agencies.

Spallanzani of Bologna (1729-1799) made his brilliant researches in 1775, which definitely and conclusively proved that generation never takes place spontaneously. His experiments were made with a large number of vegetable and meat extracts. Pieces of meat and vegetable matters were first boiled, then the extracts poured into clean glass bottles with narrow necks which were as soon sealed hermetically. They were then kept immersed in boiling water for about an hour. It was found that the infusions remained clear without forming any scum on the surface. He further showed that when the same infusion was exposed to air, it became turbid and showed presence of infusoria. In 1859, the question was again raised by Pouchet, but was finally, and for ever, settled in the negative by Pasteur in 1864, who pointed out the sources of error after repeating the experiments of Pouchet.

Apart from those observations on fermentation, and spontaneous generation, Pasteur established the *theory of molecular dissymmetry.*

His next studies were on wine, beer, etc.

His studies on diseases were first directed to a peculiar affection of silk-worm, called the silk-worm plague, which was causing havoc to the silk industry of France. Pasteur discovered that the cause lay in some infection of the worm, and the eggs were infected from the beginning He suggested segregation of the diseased worm, and thereby saved the silk industry of France.

His attention was next directed to anthrax which was having a heavy toll of the sheep population of France. He discovered the causative factors, and once more saved France from loss of the wool industry.

Pasteur accidentally found that fowls injected with old non-virulent culture of fowl cholera, resist a heavy dose of virulent culture of the same organism given subsequently, whereas control fowls die very rapidly. Thus, a great mystery was solved, and Pasteur realised the great utility of this discovery for protection of man and animal from infectious diseases.

In the meantime, **Jenner** had discovered the effect of vaccination as protection against the attack of small-pox. These wonderful discoveries of Pasteur and Jenner formed the basis of preventive treatment, and led to the discovery of inoculations against anthrax, rabies, etc. The discoveries of Pasteur have also served as the basis of suggestions which made the names of **Lister** (the Father of Aseptic surgery) and **Koch** (the Father of Modern Bacteriology) immortal.

Pasteur's self-sacrifices in his unselfish and untiring devotion to the cause of his country and mankind, elicited a strong desire among his people for the establishment of a permanent institute for researches on these lines, and an Institute called the "Pasteur Institute" was opened on the 14th November 1888 with willing contributions from every part of France This formed the nucleus, and, within 30 years of its establishment, several dozens of "Pasteur Institutes" were installed in different parts of the world with similar aims, and, no less than six such Institutes now exist in India.

The greatest curse which occasioned irreparable interruptions to the invaluable work of Pasteur, was his repeated attacks of apoplexy. The first and most severe attack which threatened the life of Pasteur was in October 1868, at the age of 40. He recovered from that, but the paralysis remained permanently. None of these calamities could stand in the way of his researches, and he continued his unceasing work until his death in 1895. His tomb is still to be seen just at the entrance of the Pasteur Institute of Paris lying by that of his wife.

The estimation of Pasteur by his people was very great. In fact, twelve years after his death, a vote was taken upon the relative prominence of the greatest Frenchmen of the ninteenth century, and Pasteur was placed at the top of the list, Victor Hugo coming next with a difference to the tune of one hundred thousand votes. The French also showed their high regard for Pasteur by affixing his figure on their postage stamps.

KOCH

Robert Koch was born in 1843. He was a bacteriologist, and the discovery of many of the most important pathogenic bacteria was reserved for him. He was a pure scientist, and his observations and tests were rigid and crucial.

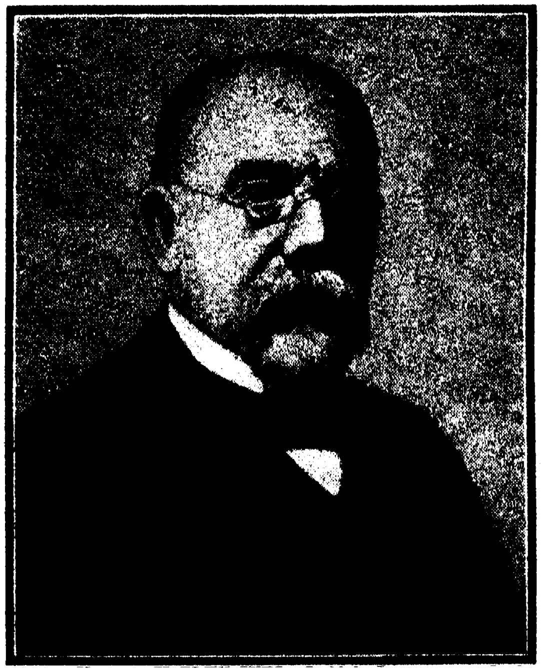


Fig. 3. Robert Koch.

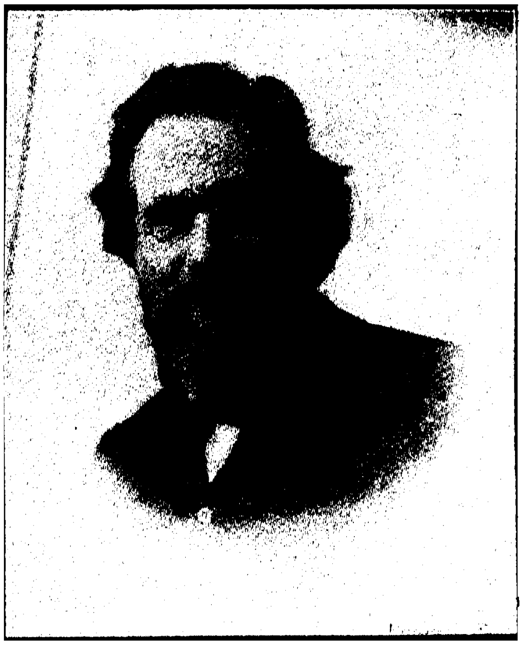


Fig. 4. Elias Metchnikoff.

His method of establishing the relation of a particular organism with a particular disease is admirable. This is known as *Koch's Postulates.* They are:—

(1) That the pathogenic organism should be found in the lesion or blood of the diseased man or animal.

(2) That the organism can be cultivated artificially in pure culture.

(3) That this pure culture will reproduce the disease when injected into another animal (which is sound).

(4) That the same organism will be recovered once more from the lesion or blood of this inoculated animal.

He observed under the microscope, by means of aniline stains, the anthrax bacillus and its spores. He also observed in 1876 the resistance offered by the spores against heat and disinfectants. His next publication "examination on the etiology of wound infection" in 1878 was still more famous. He discovered the tubercle bacillus in 1881, and the cholera vibrio in 1883. This started the foundation of modern bacteriology, and by the strict discipline of his methods, quite a number of other infectious diseases could not remain a mystery any more, some of them being diphtheria, dysentery, tetanus, pneumonia, plague, leprosy. Not only the identification of the causative organisms, but also their biological properties, and the toxins—the substances responsible for the disease-production—were revealed to the observers.

He was the first to introduce the method of examination of micro-organisms by staining them, as also to introduce solid transparent culture media for the cultivation of bacteria, whereas, before his time, only the fluid culture media of Pasteur were in use.

Koch was not satisfied with the discovery of the organism only. He worked patiently to find out some curative agent for tuberculosis, the ultimate result of his attempts being the discovery of the *tuberculins*, which has subsequently been found to be of great therapeutic and diagnostic value. His announcement, that tuberculosis is a virulent, infectious and inoculable disease, is also a very remarkable event. In his study of leprosy, he discovered that the nasal secretions contain a very large number of the organism, and that this is the earliest source of exit of the organism from the body.

In 1896 Koch was invited by the English Government to visit South Africa to investigate and control rinderpest which was causing havoc there. From this place he was deputed to India to investigate about the problem of the human plague. Here he became interested in malaria and devoted much time on research about it as well as on that about blackwater fever. He was also sent to Central Africa as the head of an expedition for the study of trypanosomiasis, where he discovered that atoxyl is a curative agent for this deadly disease.

After his return to Germany, he found that his only recreation was to plunge headlong into medical researches. One finds him every day as the first to enter the Institute, and, at the close of the day's work, the last to leave it.

The career of Robert Koch was a very brilliant one. His discovery of the causative organisms of many a dangerous disease, and his invitation by the English, French and other nations to investigate diseases in South Africa, West Africa, Egypt, India and elsewhere, made him the greatest man of his time, and *Koch's Institute* at Berlin became the centre for the study of infectious diseases. He received the Nobel Prize in 1905 and in 1906. Among his pupils we find the brilliant workers **Gaffky, Pfeiffer, Kitasato, Loeffler, Welch** and others.

METCHNIKOFF

Metchnikoff (1845-1916), the discoverer of *phagocytosis,* is the father of one of the most important theories of immunity. His monumental works are on comparative pathology of inflammation, immunity of infectious diseases, etc. He received the Nobel Prize in 1908.

EHRLICH

Paul Ehrlich is the founder of hæmatology and discovered his tri-acid stain, diazo reaction of urine, intravital staining, mast cells, etc. He divided the leucocytes into neutrophiles, etc., and improved on Behring's diphtheria antitoxin therapy by standardizing it into units. His monumental works are on dye-stuffs and his chemical explanation of immunity (the *Side-Chain Theory*, and in the discovery of *Salvarsan* in collaboration with **Hata,** his Japanese assistant. The works of Ehrlich have changed the direction of the modern medicine from the Bacterial to the *Biochemical Theory of Diseases* and their cure.

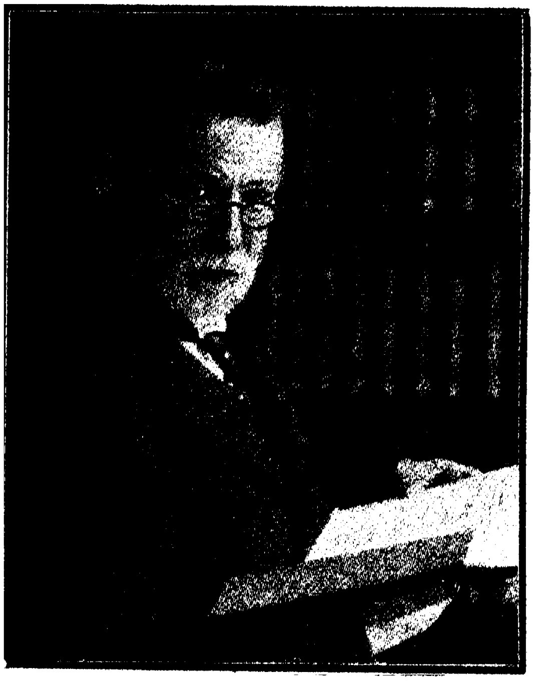


Fig. 5. Paul Ehrlich.

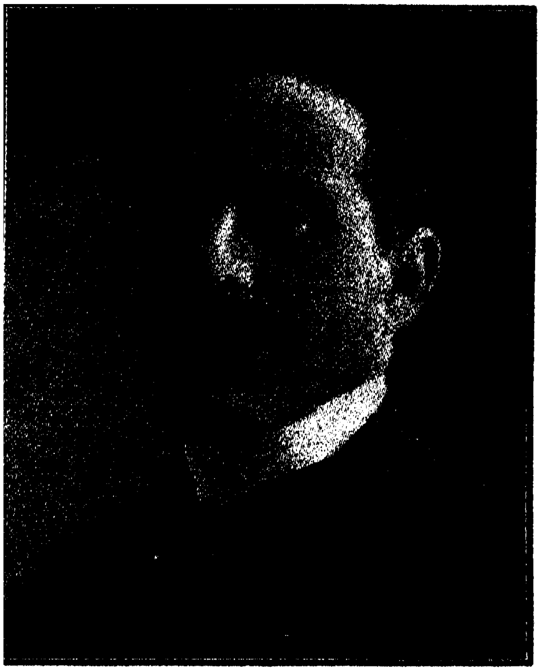


Fig. 6. Wassermann.

WASSERMANN

August von Wassermann (1866-1925) discovered the sero-diagnostic method of syphilis, known after his name, only one year after the causative organism of the disease was discovered by **Schaudinn** in 1906.

The eminent scientists who have advanced bacteriology and pathology are very numerous. The most prominent ones are **Conheim** (1839-84), **Weigert** (1845-1904), **Klebs** (1834-1913), **Loeffler** (1852-1915), **Gaffky** (1850-1918), **Wright** (1861-), **Widal** (1862-1929), **Flexner** (1863-), **Lister**

(1827-1912), **Laveran** (1845-), **Ross** (1857-), **Schaudinn** (1871-1906), **Bordet, d'Herrell,** etc.

I cannot close this chapter without mentioning the names of two Japanese workers whose names are uppermost in our minds. One of them is **Shiga,** whose recent visit to Calcutta, during the preparation of the manuscript of the Second Edition of this book, has left a very pleasing memory. The other is **Noguchi.**

NOGUCHI

Hideyo Noguchi, one of the most brilliant Japanese research workers, was born in Japan in 1876. While he was only 25 years of age, he left the Tokyo University, being invited to the post of assistant lecturer in pathology at Philadelphia. Within two years of his appointment he made himself so prominent by his original researches that he was appointed research assistant to the Carnegie Institute, and shortly after that, he joined the staff of the Rockefeller Institute.

Fig. 7. Hidyeo Noguchi.

Fig. 8. Shiga.

His researches on original lines were enormous, and these works (specially, that on spirochætes) have acquired for him an international reputation and, at the same time, offered him as sacrifice to the altar of science.

He drew his last breath in May 1928, while carrying out his investigations on yellow fever in Africa, being stricken with the disease in spite of his usual thoroughness and care, and thus joined the distinguished band of martyrs to science.

Noguchi carried on original work on serological studies, *e.g.*, agglutinins, opsonins, hæmolysins, snake venom, serological diagnosis of syphilis, etc.

Noguchi took the medical world by surprise by his successful culture of Treponema pallidum, and again by demonstrating the presence of these organisms in the brain in G. P. I. and in the spinal cord in tabes, thus proving definitely the syphilitic nature of these affections.

His investigations on Weil's disease led to the discovery of the causative organism—a type of spirochæte—which he named *Leptospira Icterohæmorrhagiæ.*

In 1918 he cultivated from the blood of yellow fever cases a leptospira, which differed from that causing Weil's disease, and he termed it *Leptospira icteroides.*.

His researches on vaccinia virus, serological and cultural relationship of various leishmania and insect flagellates, immunological reactions in Rocky mountain spotted fever, virus of Oroya fever and Verruga peruviana—Bartonella bacilliformis, on trachoma, etc, have placed him in the forefront amongst the scientists of the world.

PATHOLOGICAL TECHNIQUE

Post-mortem technique.

Virchow formulated the modern method of performing an autopsy: this consists in studying the organs *in situ,* and, after the topographical conditions are studied, each organ is examined separately both before and after its removal from the body. His predecessors—specially **Rokitansky**—used to perform the autopsy by removing all the organs from dead body and then studying each individual organ separately. **Hauser** called attention to the *freezing method* which helps a good deal in the study of topographic-pathological anatomy.

Microscopical technique.

The advancement in this line is of special importance. The science is undoubtedly indebted to **Abbe** and **Zeiss** for the development of the microscope and its component elements, but, much more, to those who helped to study, develop, and perfect the art of cutting fine sections of the tissues and staining them for finer study. **Conheim,** who has revolutionized pathology by his monograph on the phenomenon of inflammation, is the father of numerous methods in histological technique. His histological work has been carried further by his eminent pupils **Ehrlich, Weigert, Neisser, Heidenhein** and many others. To Weigert we owe the *differential staining* of the nervous system, elastic tissue, bacteria, etc., and also the *quantitative law* that in an injured tissue the amount of repair is always in excess of what is required.

Christeller's *freezing method of cutting sections of whole organs* is a distinct advance in histo-topographic studies.

The foremost of those to be mentioned under this heading are **Weigert, Ehrlich, Unna, Michaelis, Goldmann, Pappenheim** and many others. Histology of the nervous system owes its existence to **Weigert, Nissl, Spielmeyer, Golgi, Ramon y Cajal, Bielschowsky** and others.

Although it is not definitely known whether the staining re-actions depend on chemical or physical (adsorption) processes, still the clearness, the sharpness, and the beauty of the staining methods are very much appreciated.

The histologists were not satisfied simply by staining each individual tissue element as well as their component elements which allow themselves to be stained, rather specifically, but they also studied them during their life, and that, with full activity—the so-called vital and supravital staining.

The subject of tissue-culture, formulated by **Harrison** and **Carrel,** has greatly facilitated the study during the growth of different types of cells and tissues, and, the effect of various chemical, physical and other influences on them.

The researches and teaching on **Immunity Reactions** started from the works of Pasteur, Buchner, Koch, Ehrlich, Metchnikoff, etc. The two main theories of Immunity are those of Ehrlich and Metchnikoff, the details of which are discussed elsewhere. The chemical and physico-chemical studies of the immunological processes are due to Ehrlich, Bordet, Madsen, Arrhenius and others, while the practical application of these processes in the diagnosis of different infectious diseases are the result of the studies of Bordet, Gengou, Widal, Wassermann, etc.

CHAPTER II

HOW TO STUDY PATHOLOGY

Definition.—*Pathology* is the science which deals with the study of diseases.

The study of Pathology extends over a wide area in the field of medical science. Pathology is generally treated under two main divisions:—

(1) **General Pathology**—which is the science leading to the study of the causes of the diseases, the structural and functional changes of the tissues in the diseased conditions, and the course, progress, and termination of diseases.

(2) **Special Pathology**—which is the science which deals with the abnormal conditions in the individual diseases, including—

(*a*) Surgery, or external pathology, and

(*b*) Medicine, or internal pathology,

which, in their turn, are subdivided into special subjects—Gynæcology, Ophthalmology, Dentistry, Laryngology, Otology, Rhinology, Dermatology, etc.

Let us now confine ourselves to the study of *human pathology*, that is, the study of human beings in their diseased conditions, and leave, for the present, the study of *comparative pathology* or the study of diseased processes in animals other than human beings.

One desirous of studying the human being in diseased conditions must possess clear ideas about the normal structure and functions of human body. Thus, a fair knowledge of anatomy and physiology is essential before one can proceed to the study of pathology. Study of physiology, again, requires, a sound knowledge of chemistry, physics and biology.

The study of pathology can be conveniently divided into the study of *clinical pathology* and *laboratory pathology*. The former requires continual observation of the picture manifested by the diseased process; and the more one can develop the power of observation, the more completely and easily one can master the subject. The study of laboratory pathology, again, requires continuous observation of the gross and minute structural changes, as well as the disturbances of functions in diseased conditions. Artificial production of a disease in lower animals—known as animals experiment—and, a study of the changes, structural and functional, produced in them, also form a part of the study of the subject of laboratory pathology.

Experiments must be preceded by observations, and clinical observations must precede laboratory observations. Here, again, no sharp line of demarcation can be drawn between the clinical study and the laboratory study. The hospital might be regarded as a laboratory, and the laboratory is only an extension of the wards, and both serve the purpose of the study of the diseased condition. (*see* the definition of pathology).

For the study of pathology, the beginner requires a teacher first and, then, the book. He should first go to the surgical wards to study the gross pathology or external pathology, and, at the same time, to develop the power of observation which is the secret of future success and which is only obtainable through the help of the teacher. For instance, he goes to the wards one day, and probably finds a patient with a swelling on his arm. From his previous observations on his own person as well as on other normal ones, he at once recognises it to be an abnormality, and, naturally, asks his teacher about the nature of the abnormality. He learns from his teacher that it is an abscess, and, at the same time, observes through his aid that the affected part is not only swollen, but is also red, painful, hot to the touch, etc.

In another bed the student will probably meet with a patient with a swelling, which, on enquiry, he learns to be a fibroma. He will at once realise that it is not an abscess, from the absence of the characteristic signs. Thus, he will come to know that all swellings are not abscesses, and he will gain the power of differentiating diseases.

After a few days, he probably finds that the abscess which he observed in the first case, has become soft, but is still painful. He then enquires why the abscess, which was hard before, has softened. He then observes that the patient is put on the table, and the abscess incised, and a quantity of peculiar fluid comes out, which, on enquiry, again, he learns to be pus. Subsequent observation will show that, along with the evacuation of the pus, the pain, fever and other distressing features have all disappeared. He then enquires, and learns, the relation of fever, pain, etc., with the abscess before and after the drainage. His teacher may demonstrate to him the causative organisms under the microscope by appropriate staining of the pus, and the student thus learns the etiology of this abnormal condition.

After dressing the wound for a few days, the student will observe that all purulent matter has been cleaned off, and the base of the wound is granular and bleeds easily. On enquiry, he learns the process of healing of wounds, and the nature of the granulation tissue. In course of time, he will see that the whole wound has become filled up, and has healed completely. He will, in this manner, study all the cases of the ward, the pathological processes of which can be studied with the naked eye. In cases of deep-seated pathological processes, he will also be able to study them on the operation table, as has been said by eminent surgeons in cases of obscure abdominal affections "open the lid and see."

He will next come to the medical wards to study internal pathology. Contrary to his experience in the surgical wards, he will not be able to see the pathological changes directly with the naked eye. For instance, in a case of pneumonia, he learns that there is a gross lesion in the lungs. He is absolutely helpless if he tries to study the disease by direct observation of the lungs. He shall have to rely on some signs and symptoms, which he will observe under the instruction of his teacher, and will, ultimately, be able to mark off definitely the areas of the lungs affected by the disease.

Here comes the relation of the ward and the laboratory. In the ward, he has only to depend on his previous observations, so as to read the different signs and symptoms and to interpret them correctly. This is occasionally supplemented by the use of some instruments, *e.g.*, sphygmomanometer, etc. Similarly, he will also be helped by the laboratory findings of leucocytosis and pneumococcus. Thus, the ward and the laboratory come to mean the same thing so far as the study of diseases is concerned.

This is the study of pathology in the *living*. Then comes the study of pathology in the *dead*.

Suppose the man suffering from pneumonia is dead, and the student gets the opportunity of witnessing the post-mortem examination. He will find that the post-mortem examination will confirm his observations during life. He will then proceed with the study of the materials obtained from the post-mortem room both with the naked eye and under the microscope.

The naked eye examination, or, the study of *morbid anatomy*, is greatly facilitated by the study of the museum specimens, which means the collection of a large number of similar post-mortem materials.

The microscopical examination, or, the study of *morbid histology*, requires a laboratory properly equipped with the materials for cutting sections and staining them.

He may further investigate into the nature of the causative organism, for which he requires the bacteriological laboratory. He may extend his study by experiments on laboratory animals, in which he may reproduce the disease and study, at will, all the changes he observed by the study of human pathology in the living and the dead.

For the study of the complex processes, *e.g.*, immunity and other functional disorders, or, the study of *morbid physiology*, biochemical, serological and other laboratories are essential.

Thus, the student will commence his study in the surgical wards, then he will proceed to the medical wards, post-mortem room, pathological museum, and histological, parasitological, serological, biochemical and other laboratories.

The study of clinical pathology and that of laboratory pathology must go hand in hand. One cannot be a successful clinician without a thorough knowledge of laboratory pathology, and *vice versa.*

After one has got a prolonged experience in the laboratory methods with extensive preliminary clinical observations, one shall select a special subject for thorough study, as it is almost impossible for one man to master the whole field of knowledge in the subject of pathology. He may select the clinical subjects, such as gynæcology, ophthalmology, dermatology, etc., or the laboratory subjects, such as bacteriology, protozoology, helminthology, histology, serology, biochemistry, etc.

CHAPTER III

HEALTH AND DISEASE

The human body, with all the cells and intercellular materials, traces its origin from a single cell—the fertilised egg-cell, which, in its turn, is formed by the union of a single spermatozoon with a single ovum. This fertilised egg-cell divides into 2, 4, 8, and so on, until millions of cells are produced, each having some particular function to perform. Thus, some turn out to be epithelial cells, some connective tissue cells, etc., with the production of all the component elements of the human body. It has been said that the individual spermatozoon of a particular male, and the individual ovum of a particular female, differ, to a great extent, from that of another male or female, and, at the same time, the influences affecting the fertilised egg, either in the uterus or outside it, though apparently the same, are never identical for two individuals. Thus, it is quite usual to find that no two human beings are exactly alike.

From these facts it would be quite clear how difficult it is to determine what is to be regarded as **the normal individual.** Thus, the standard of 'normal' is a very difficult matter to decide. For the sake of convenience, scientists have determined the standard of health by observations of large number of so-called healthy individuals, and the average of the series, with certain limitations, is regarded to be the *normal,* beyond which it is *abnormal.*

Thus **disease** may be defined as "an abnormal state of the living body, characterised by an alteration of structure or by a disturbance of function."

SCOPE OF PATHOLOGY

Pathology deals with the following:—

(*a*) Etiology, or causes of diseases.

(*b*) Structural and functional changes of the body. These are included under the terms pathological anatomy, pathological histology and pathological physiology, the first two indicating gross and minute structural changes. They are also termed morbid anatomy, morbid histology and morbid physiology, respectively.

(*c*) Progress of diseases.
(*d*) Termination of diseases.

TERMS USED TO DESCRIBE MORBID PROCESSES

(1) According to the Causes and Distributions of the Diseases

Infectious—when the disease is caused by living organisms, either vegetable or animal, such as fungus, bacteria, protozoa, worms, insects, etc. (For Koch's Postulates, *see* Page 23.) Specific—when it is caused by a particular species of living organism, and by no other. For example, tuberculosis is always caused by a particular form of micro-organism, B. tuberculosis. Hence, this is a specific infectious disease; whereas, an abscess, though an infectious disease, may be caused by many different species of organisms, and hence, non-specific. Toxic— when a disease is caused by poisonous substances which may or may not be derived from infective agents. Contagious—when an infectious disease can be communicated from one individual to another by contact or touch. Idiopathic—when the cause of the disease is unknown. Sporadic—when a disease occurs in isolated areas at irregular periods. Epidemic—when a disease occurs in a large number of men at one time in a particular locality. Endemic—when a disease is always present in a locality. Pandemic—when a disease occurs simultaneously throughout the world. Hereditary—when a disease is derived from one or both parents. Congenital—when a disease is present at birth, but may or may not be hereditary. Acquired—when a disease is contracted after birth. Infantile—when occurring in childhood. Familial—when occurring in more than one member of a family. Senile—when occurring in old age. Acute—when the onset of the disease is sudden, and of short duration. Chronic—when the onset is insidious and of prolonged duration. Subacute—when the course is neither short nor long. Primary—when the disease is occurring for the first time. Secondary—when the disease is derived from some other similar primary disease. Recurrent or Relapsing—when there is a tendency to recur. Intercurrent—when a disease develops during the course of another.

(2) According to the Structural and Functional Changes of the Body

LESIONS—are the structural changes caused by a disease. ORGANIC—when there are definite lesions of the tissues and organs. FUNCTIONAL—when there is no demonstrable lesion of the tissues and organs. LOCAL—when affecting a particular portion of the body. GENERAL—when affecting nearly the whole body. CONSTITUTIONAL—when there are disturbances of the constitution of the individual.

(3) According to the Progress of the Disease

INCUBATION PERIOD OF THE DISEASE.—This is the period, during which the infective organisms, subsequent to their entry into the body, multiply without causing any definite symptoms. PRODROMES.—These are indefinite and vague symptoms, often found during the incubation period. ONSET OR INVASION – the first manifestation of definite symptoms. ACME OR FASTIGIUM—the period of maximum activity of the disease. DECLINE—the subsequent fall of the active process of the disease. CRISIS—when the decline is rapid, with sudden disappearance of the symptoms. LYSIS—when the decline is gradual with slow disappearance of the symptoms. CONVALESCENCE—the stage of recovery from the disease. RELAPSE—reappearance of the symptoms and repetition of the disease. RECRUDESCENCE—any exacerbation of the disease. COMPLICATION—when there are some associated conditions, in addition to the usual symptoms. SEQUELÆ—are secondary diseases occurring after recovery from original primary affections; for example, gall stone originating after an attack of typhoid fever.

(4) According to the Termination of Diseases

HYPERTROPHY—increase in size and functional activity. ATROPHY—decrease in size and functional activity. REPAIR—regeneration and replacement of the lost tissue. SENESCENCE OR SENILITY—the state of being old. DEATH—the cessation of life.

CHAPTER IV

THE ETIOLOGY OF DISEASES

As mentioned before, the human body is composed of a large number of cells which originate from a single cell, known as the fertilised egg-cell which, in its turn, is derived from the fusion of two cells, one derived from the father (the spermatozoon) and the other derived from the mother (the ovum). This fertilised egg-cell must reside for some particular period in the uterus of the mother, from which it comes out by the process known as labour, and then leads an independent life. According to the stage of development, the periods of life of the human individual are known as—

Prenatal or Antenatal—before birth.
Natal or Parturial—during birth.
Post-natal—after birth.

The factors causing disease may be present either in the spermatozoa, or in the ovum, or in both, before the egg-cell is fertilised, or may be present after the fertilisation has taken place.

The Factors causing disease may be grouped as follows:—

1. **Hereditary**—when derived from what is transmitted by the ovum or spermatozoa.
2. **Acquired**—when originating subsequent to the fertilisation of the egg-cell.

The disease may be acquired—

(*a*) before birth, (*b*) during birth, (*c*) after birth.

Congenital affections must not be confounded with hereditary affections. "Congenital" means simply that at birth the individual possesses or lacks certain attributes, while "hereditary" means only ancestral qualities transmitted to the sex plasm.

Thus the terms "hereditary syphilis" and "hereditary tuberculosis" are misnomers as these are always the result of disease acquired by the fœtus *in utero* and hence are "congenital."

Congenital and hereditary affections can be divided into the following groups:—

(A) Some Infective Agent Affecting One or Both the Germ cells.

This is commonly illustrated in the case of syphilis. The spirochætes may infect either the spermatozoon or the ovum, and transmit the disease to the offspring.

In case of tuberculosis, it is very doubtful whether the infection can be transmitted through the spermatozoa. It may be transmitted through the ova, or through the placental circulation. But in the case of infection through the placental circulation, it does not come under heredity, because it takes place after the fertilisation of the egg-cell. In a majority of instances of tuberculosis, the *tendency* is hereditary, and not the disease itself.

(B) Simple Predisposition to some Particular Disease.

Like tuberculosis, carcinoma is also found to occur in some families, the members of which may show a tendency to die of cancer at a certain age.

(C) Family Peculiarities.

It is not uncommon to find some peculiarity—*e.g.*, talipes, or some other deformity—to run in the same family, being transmitted from the parents to the children. In some instances, defects in the development of any part of the body are also transmitted to the offspring.

(D) Hereditary.

This is illustrated in the instances of hæmophilia in which the males of a family do not transmit the disease. The disease is transmitted by the unaffected daughters to their sons. The males are the sufferers, but the disease is transmitted through the females.

ACQUIRED CAUSES

1. Diseases Acquired Before Birth

They may be caused by—

(*a*) defects of development, (*b*) mechanical causes, (*c*) diseases of the placenta, (*d*) diseases acquired through the mother.

(A) Defects of Development.

These are generally called *malformations*, and occur while the embryo is very young, generally before the third month. Malformation is an alteration occurring during the development of the fœtus. When the deformity is pronounced, it is called a *monster*, while less conspicuous deviation affecting only circumscribed

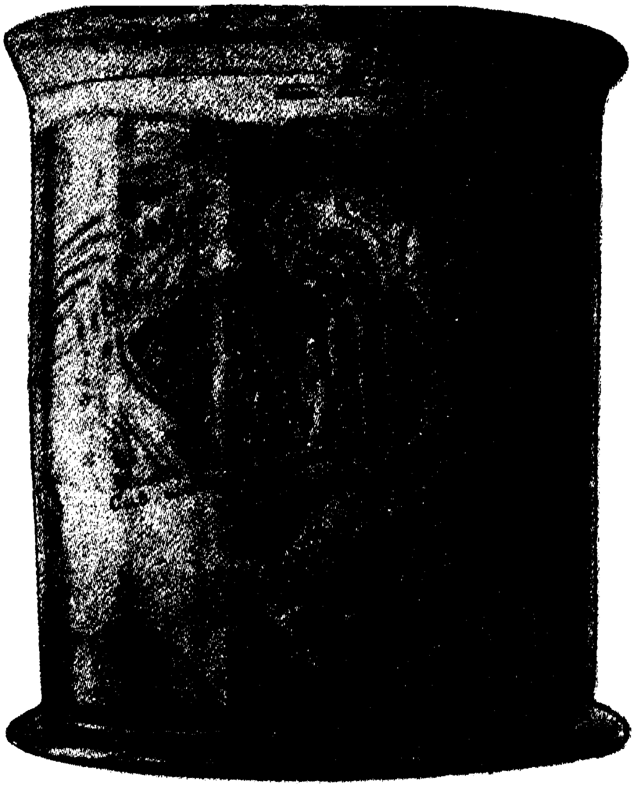


Fig. 9. Lithopædion.
(From Dr. Kedar Nath Das)

portions are termed *anomalies*. The causes which lead to the occurrence of malformations are not definitely known. It has been found possible to develop malformations artificially, in lower animals, by rough manipulation of the egg, and thus disturbing the relative position of the component cellular elements. The malformations often become so extensive as to be incompatible with life, and subsequent premature expulsion of the fœtus takes place (*abortion*). Occasionally the dead fœtus is retained inside the uterus, and becomes mummified, or, with the subsequent deposition of calcareous particles, results in the formation of a *Lithopædion*.

The common malformations are classified by Birnbaum as follows:—

1. Malformations due to arrest of development—

(*a*) Amniotic or amniogenic malformations.—These are due to anomalies of the amnion, *e.g.*, abnormal adhesions or band formation, defect of the amnion and hydramnios. The malformations are (*i*) anenecephalic monsters, (*ii*) hydrocephalus, (*iii*) symelia (the lower extremities united into a single fish-like structure), (*iv*) curvature of the spine, (*v*) malformations of the limbs, *e.g.*, spontaneous amputation, etc.,

(*b*) Agenesia or aplasia of organs or parts of the body.

(*c*) Malformation due to the arrest of development in the form of fissures or duplicatures, *e.g.*, hare-lip, cleft palate.

2. Malformations due to excess of development. This is exemplified by supernumerary mammary glands (polymastia), spleen, fingers and toes, vertebræ, etc.

3. Malformations due to errors of development, *e.g.*, transposition of viscera.
4. Malformations due to displacement of tissues and persistence of fœtal structures, *e.g.*, teratomata, dermoids and cysts.
5. Malformations due to fusion of the sexual characters, *e.g.*, hermaphroditism.
6. Double malformation, *e.g.*, union of fœtal bodies with one another which may be united only by the placentæ or umbilical cords.

The chief varieties of monsters are:—

Pygopagus—The twins are usually united in the pelvic region.

Ischiopagus—They are united in the region of pelvis, the axes of the bodies forming an acute or obtuse angle.

Dicephalus—The bodies are united, leaving the heads separate.

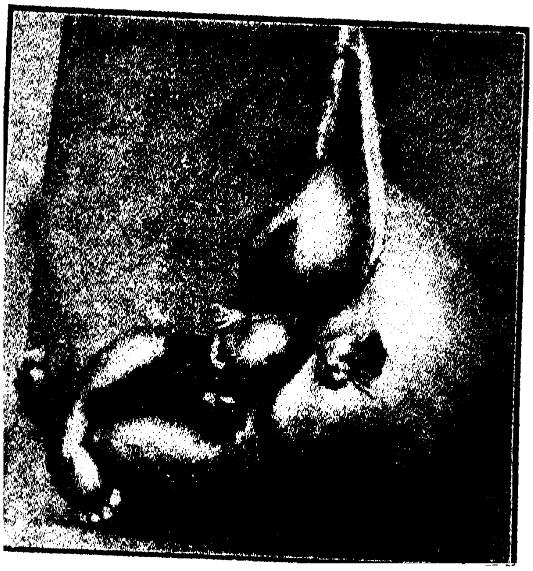


Fig. 10. Anencephalic Monster (From Carmichael Medical College Museum.)

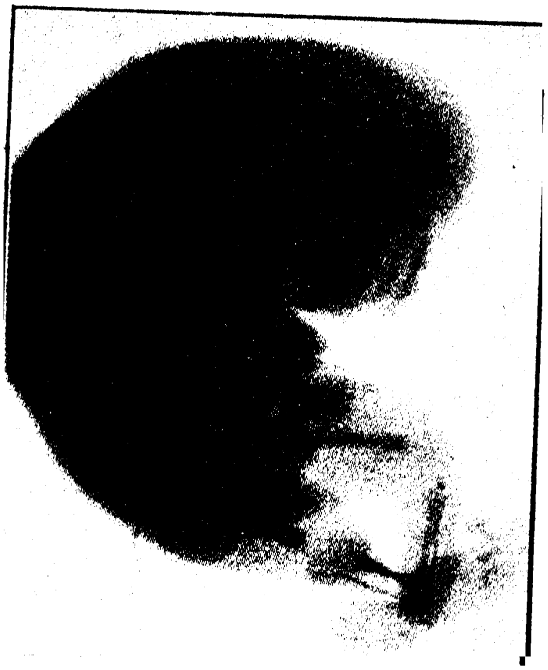


Fig. 11. Skiagram of the same Specimen shown in Fig. 10.

Diprosopus—Only the face remains double, but otherwise the bodies are completely united.

Craniopagus—Complete union except the brain.

Cephalothoracopagus—Complete union of the head, but separate thoracic and abdominal cavities.

Thoracopagus—The union is only by the thorax.

Epignathus—A parasitic shapeless mass springing from the oral cavity.

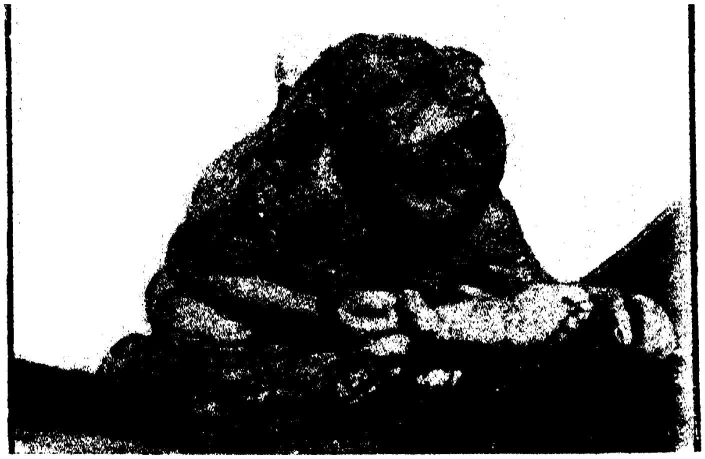


Fig. 12. Anencephalic monster.
(From Carmichæl Medical College Hospital.)

Triple or Multiple Monsters—When more than two bodies are united.

Acardiac Monster—Monster without a heart; nearly always found in twins.

Acardiac Acephalic Monster—Monster without the head, thorax, heart, lung, liver, upper extremity.

Anencephalic Monster—Defective development of bones of the cranium, resulting in the protrusion of the brain and the meninges.

Meningocele—Protrusion of the meninges only.

Spina Bifida—Defective development of the spine, generally the posterior aspect of the lumbo-sacral region as a result of defective closure of the cerebro-spinal canal.

Supernumerary Structures—These are commonly found in the case of fingers, spleen, etc.

Transposition of Viscera—The heart is present on the right side, liver on the left, spleen on the right, and so on.

Deformities—Talipes, etc.

Absence of Viscera or Openings—*e.g.*, imperforate anus, congenital absence of bile duct, absence of the transverse colon, etc.

Hermaphroditism—The presence of both male and female sexual organs in the same individual.

(B) Mechanical Causes.

Mechanical violence occasionally leads to deformity, or even death, of the fœtus.

(C) Diseases of the Placenta.

(D) Diseases Acquired through the Mother.

These may be—

(*i*) Infection, *e.g.*, typhoid, syphilis, small-pox, etc.

(*ii*) Intoxication, *e.g.*, alcohol, lead, etc.
(*iii*) Malnutrition, *e.g.*, wasting diseases, anæmia, etc.

2. Diseases Acquired During Birth

These are generally mechanical. They may be—

(*i*) deformities and injuries due to difficult labour, (the so-called obstetrical injuries);
(*ii*) infection of the child from the maternal passage, *e.g.*, ophthalmia neonatorum;
(*iii*) injury to the umbilical cord.

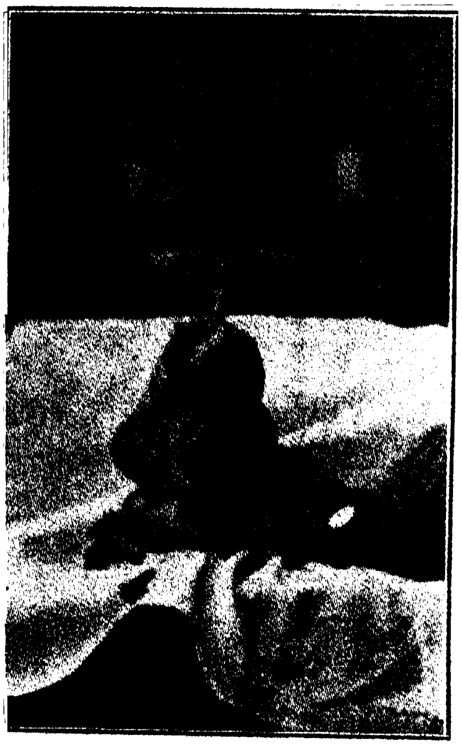


Fig. 13. Meningocele.
(From Mr. Subodh Datta)

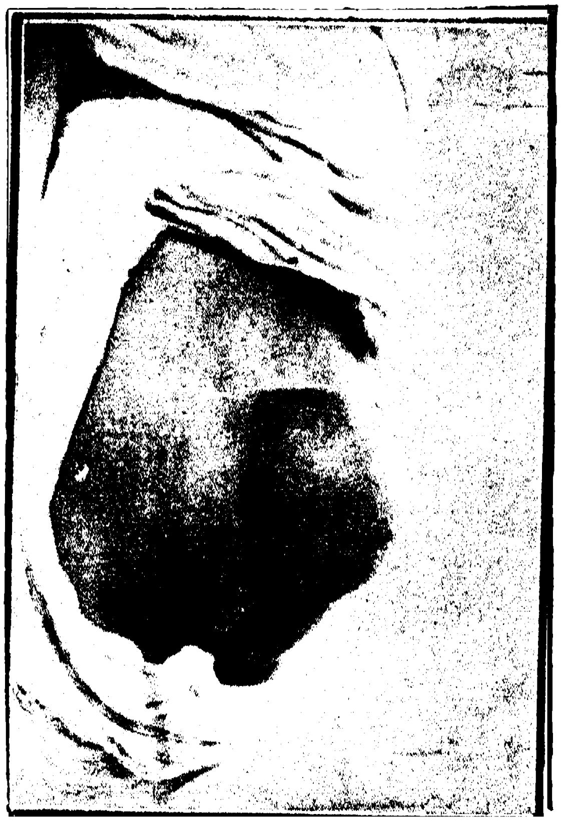


Fig. 14. Meningocele.
(From Carmichæl Medical College Hospital.)

3. Diseases Acquired After Birth

(A) Indirect or Predisposing Causes

Age.—It is an established fact that age has great influence on the problem of the etiology of diseases. Some diseases particularly affect children; others again, are the diseases of old age. Anxiety, worry and exposure to various conditions are important factors in the affections of early adult life. Children are predisposed to various infectious diseases, (*e.g.*, measles, whooping cough, etc.), more than adults. In early adult life, physical and mental strains as well as liability to various infections and intoxications predispose to diseases.

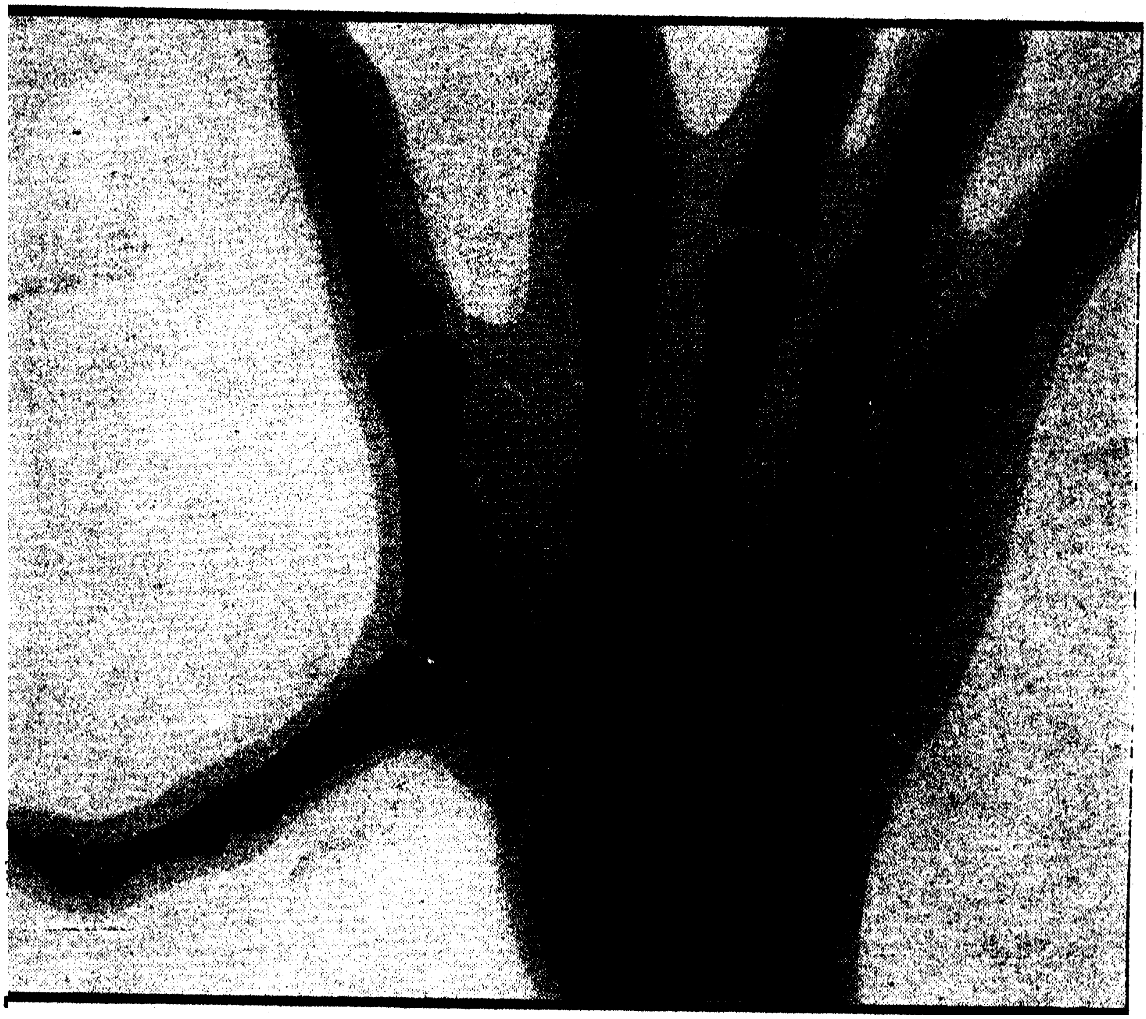


Fig. 15. Supernumerary finger originating from the thumb.

In old age, the atrophy of all the functional elements of the body, especially of the generative organs and of the organs of nutrition, predisposes them to various diseases. The liability to cancer increases with age, as well as to fibrous and other degenerative changes of blood vessels and other organs.

Sex.—Up to the period of puberty, there is generally no difference as regards special predisposition due to sex. After puberty, the special structural and functional differences of the organs of the females render them liable to the consequences of pregnancy and childbirth. Carcinoma, again, is relatively commoner in the female than in the male.

Occupation.—Occupation involving over-exertion predisposes to various diseases, on account of the increased demand made by the muscular tissue for nutrition which is carried out by the organs of circulation. When the nutrition is not readily and sufficiently available, or when the strain on the muscle and circulatory organs is excessive, various diseases will invariably result. Disuse, on the other hand, leads to atrophy and degeneration of the organs.

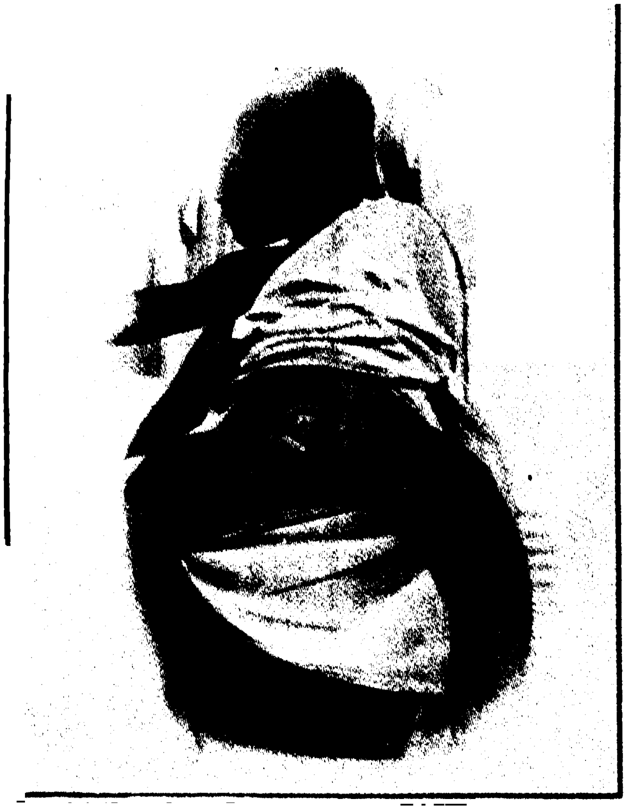


Fig. 16. Ectopia Vesica. (From Carmichæl Medical College Hospital.)

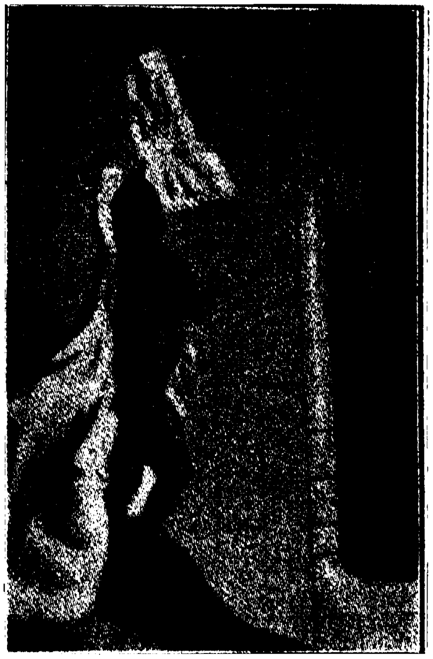


Fig. 17. Exomphalos (Congenital umbilical hernia). (From Mr. Subodh Datta).

Race.—Some races are predisposed to some affections, while others are immune to various diseases. Jews are said to be resistant to tuberculosis but predisposed to diabetes.

Climate.—Pneumonia, frost-bite, etc., are more common in cold climates, whereas sunstroke and gastro-intestinal affections are commoner in hot climates.

Season.—Respiratory diseases, *e.g.*, bronchitis, laryngitis pneumonia. are more common in winter, whereas diarrhœa.

especially of children, and other infectious diseases are more common in summer.

(B) Direct Causes

These are (1) Nutritional disturbances; (2) Mechanical disturbances; (3) Thermal disturbances; (4) Electrical disturbances; (5) Exposure to light and noise; (6) Chemical causes which last are sub-divided as follows:—

(*i*) Inorganic. (*ii*) Organic—(*a*) vegetable; (*b*) animal.
(*iii*) Metabolic (auto-intoxication).
(*iv*) Infectious—

(*a*) mycotic;
(*b*) bacterial;
(*c*) protozoal;
(*e*) verminous;
(*d*) helminthic;
(*f*) unknown.

(1) Nutritional Disturbances

These are disturbances in the quantity and quality of the nutritional elements required for the healthy organism as measured in terms of calories. The amount of calories, again, depends on the amount of work done by the individual, and, also, by external agencies, *e.g.*, climate, etc. Generally speaking, a healthy individual requires about 3,000 calories daily. Rubner gives the amount of calories required by an intellectual weighing 60 kilograms as 100 grm. protein=410 calories; 50 grm. fat=465 calories; 400 grm. carbohydrate=1,640 calories.

The diet must contain proportionate amounts of these three proximate principals; otherwise, too much excess of any one of them, *e.g.*, protein, will lead to diseased condition, *e.g.*, gout.

Inanition is the term applied to the condition of fasting for a fairly long time which may even lead to death. One can fast for one or two days without any great disturbance of health.

Deficiency of vitamins in the diet will produce what Funk describes as "*avitaminosis.*"

Vitamins are generally of five forms:—

(*a*) Vitamin A which is soluble in fat. Deficiencies of this in food will produce rickets, xerophthalmia, calcium phosphate urinary calculi, etc.

(*b*) Vitamin B, which is soluble in water. Deficiency of this will produce beriberi (polyneuritis).

(*c*) Vitamin C—the antiscorbutic vitamin.

(*d*) Vitamin D. Deficiency of this produces pellagra and hunger œdema.

(*e*) Vitamin E. Deficiency of this will disturb the normal function of reproduction.

Mineral substances, especially iron and calcium, also play a very important part in nutrition. Iron and calcium are required for the blood-forming organs and the skeleton respectively; and their deficiency will lead to anæmia, rickets, Barlow's disease, etc. Similarly, other substances, *e.g.*, sodium chloride, water, iodine, oxygen, etc., are indispensably necessary for the maintenance of life.

Deficiency of oxygen-intake results from—

(*a*) Insufficiency of oxygen in the inhaled air, as, in closed, smoky, or otherwise less oxygenated chamber, high altitude, or, during intra-uterine life with loosened placenta.

(*b*) Obstruction to the passage of air into the body by causes existing within the lumen or in the wall of the air passages, or pressure on them by tumour. aneurism, enlarged thyroid or such conditions as hanging, throttling, etc.; and lastly,

(*c*) Quantitative or qualitative deficiency of the oxygen carrier—the red blood corpuscles.

Suffocation means death due to sudden obstruction to the passage of oxygen. *Asphyxia* is death due to slow obstruction to the passage of air. Blood and tissues become overcharged with carbon dioxide. *Dyspnœa* is the condition produced by deficiency of oxygen and characterised by deep rapid breathing with working of the accessory muscles of respiration. *Cyanosis* is the bluish colour of the tissue when the blood cannot regain its normal oxygen content.

(2) Mechanical

These are generally of the nature of *Traumatism* and *Pressure*.

Traumatism.—There are infinite varieties of traumatism met with in the causation of diseases. Civilization, with its invention of new destructive machinery of warfare as well as of everyday workshop, furnishes new everchanging varieties of wounds caused by them.

The lesions depend mainly upon the nature of the instrument, whether blunt or pointed, its composition, its velocity, and the resistance offered by the tissue. When the violence is slight, there may not be any lesion whatsoever. When the violence is greater, it may produce a bruise, with rupture of minute vessels. When it is still more severe, it may cause laceration of the soft parts, fracture of the bones, dislocation of joints, or rupture of internal organs.

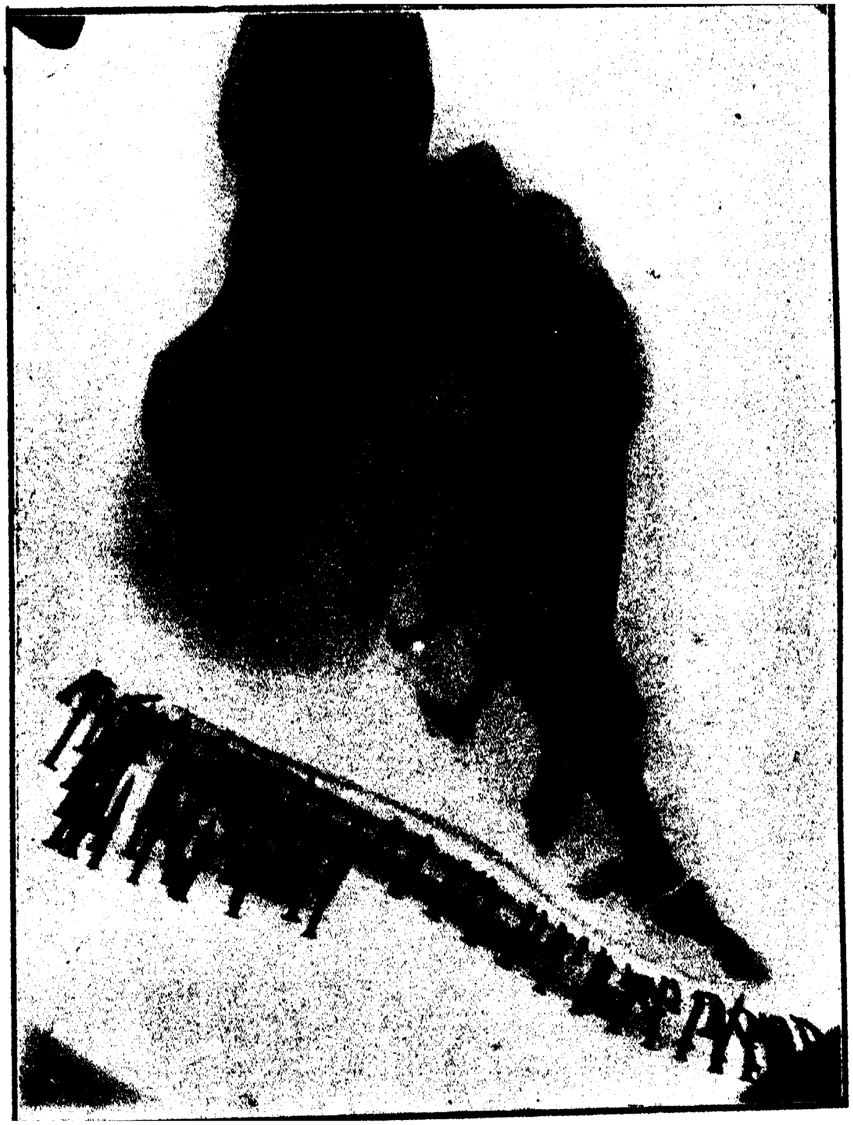


Fig. 18. Foot of a Chinese Lady.

If the affected tissue is rich in blood supply, there may be extensive hæmorrhage due to rupture of blood vessels, which may terminate fatally.

A fracture may be a simple or a compound one. In the latter instance, when the fractured bone projects through the lacerated skin, and is exposed to infection from outside, serious consequences may follow. According to the extent of the damage done, a fracture is known as complete, partial, green-stick, fissured, impacted, comminuted, etc. In cases of fracture and operations on long bones, dislodgment of the fat cells from the bone marrow occurs very easily, and may lead to fat emboli of the lung. When the fragments are in apposition, without any soft tissue intervening, the process of healing takes place early, resulting in the formation of a *callus.* Injuries of the skull often lead to a greater damage at the point opposite to the seat of injury. This is known as *contrecoup.* These injuries may be incised, lacerated, contused or perforated.

During intra-uterine life, the fœtus is amply protected, on account of its position and surrounding media, from ordinary violence on the part of the maternal organs, but when the violence is severe, abortion, or, one or more of the many different forms of malformations, may take place.

Pressure.—Any pressure applied continuously for a long time interferes with the circulation of the area, and subsequently results in the *atrophy* of the part. On the other hand, pressure, applied intermittently, and thus allowing the circulation to be maintained during the period of removal of the pressure, leads to *hyperplasia* of the part. Common examples are corns and other thickenings of the skin. The effects of continued pressure are illustrated by the deformed feet of Chinese ladies, the constriction resulting from wearing tight clothing, corset, etc.; pressure effects of tumours or aneurisms may also be cited as examples.

When the pressure is very violent, it often leads to extensive damage to the tissues by its constricting effect and may be fatal.

Human beings and animals can bear the pressure of 5 to 7 atmospheres, but only when the compression and de-compression occur very slowly. The effect of compression is mainly felt in the ears, because of the resulting difference in the pressure on the two sides of the tympanic membrane. This difference may be sufficient to lead to rupture of the membrane. The tone of the voice also alters as the pressure rises, and at a pressure of 3 atmospheres whistling and speech become impossible. The skin looks extremely pale from the compression of the blood vessels. The pulse rate is diminished (by about 15 beats) with 1 to 3 atmospheric pressure. The most important factor is the increased solution of gas in the blood, which becomes more and more saturated with gases, mainly nitrogen

Sudden relief of pressure leads to sudden expansion of gases dissolved in the blood, with formation of numerous bubbles.

These bubbles act as emboli. It has been estimated that by sudden decompression, 1,700 ccm. of nitrogen can be separated from blood. This form of mechanical disturbance occurs in the so-called Diver's or Caisson disease. A diver working at a depth of 60 feet must take at least 40 minutes to have decompression; otherwise, pains in the muscles, paraplegia, disturbances of the circulation and respiration or even death may occur.

Low atmospheric pressure, *e.g.*, in the high altitudes (as in an aeroplane), also affects the human organism. Under these conditions, other factors, *e.g.*, humidity, movements and the electric conditions of the air, effect of the sun, etc., also play very important part.

The main changes take place in the blood. Increase in the number of the red cells (*polycythemia*) occurs as also increased capacity to absorb more oxygen (*hyperglobinæmia*). Here, again, with sudden changes of pressure, *e.g.*, in an aeroplane, various symptoms occur.

(3) Thermal

This is either heat or cold.

The effect of heat can be easily studied on living tissue either on a slide or in a capillary tube. When the temperature is gradually raised (up to 40°C =104°F) as in some fevers, the cells accommodate it quite well and the leucocytes can be seen actively moving. At 50°C (122°F) all the cells die. The leucocytes become fixed and the red corpuscles show irregularities and constrictions, with formation of bud-like processes which separate ultimately. At 65°C (140°F) the hæmoglobin comes out of the red cells (hæmolysis), and at 70°C (157°F) coagulation of the blood takes place.

Burns.—According to the intensity and duration of the action of heat, the tissue may be rendered red, blistered or actually roasted. In case of severe burns, there is extensive drainage of fluid from the system through the burnt area. The blood is concentrated thereby, and this often leads to coagulation of blood inside the vessels (thrombosis). It is generally believed that duodenal ulcers following extensive burns are due to the thrombosis of the vessels of the duodenum, resulting in malnutrition and necrosis of the area supplied by the blood vessels. The necrosed area may be subsequently digested by the digestive ferments of the stomach, with the formation of ulcers.

Death generally follows extensive burns. It was formerly believed to be due to the absorption of a large amount of toxin from the affected area, but it has been found that the burnt tissue is not toxic, so that death is now believed to be due to shock.

Heat affects the human tissue (*a*) by radiation (sun, artificial light, etc), (*b*) by contact with gases, vapours or fluids at abnormally high temperatures, and (*c*) by contact with solid substances at a high temperature (red hot iron). According to severity, burns are divided under six grades or degrees:— (1) The first degree burn is characterised by inflammation of the superficial layers of the skin, with formation of no blisters (*erythema*). (2) The second degree burn is that in which there is inflammation of the skin, resulting in detachment of the epidermis and formation of blisters. (3) The third degree burn constitutes partial destruction of the dermis. (4) In the fourth degree burn, the whole thickness of the dermis is destroyed. (5) In the fifth degree, sloughing of the soft parts takes place. (6) In the sixth degree, the burnt tissue is carbonised.

Heat Stroke and Heat Exhaustion.—These occur when the atmospheric temperature is very high, specially when there is an abundance of moisture. These generally take place with people of weak constitution. Hedginger calls attention to the fact that persons with imperfectly developed chromaffin system are the usual sufferers.

Sunstroke or Insolation.—This is due to the exposure of the body, especially the head, to the prolonged action of sun's rays.

Cold.—Exposure to cold produces contraction of the vessels of the part, so that the skin becomes pale and bloodless. The metabolism is greatly diminished, and in extreme cases, may produce gangrene, which becomes either dry or moist according to the amount of blood present in the area. Examples are frostbite, chilblain, trenchfoot, etc.

(4) Electricity

Lightning or discharge of powerful electric currents may cause immediate death. This is either due to the resistance offered by the body to its passage or to the production of shock and injury to the nervous system.

X-ray or radium causes dermatitis, mainly on the constantly exposed parts, which may ultimately develop into carcinoma.

Radio-dermatitis.—X-rays, radium and other radio-active substances give out radiation which are neither felt nor seen, but injudicious exposure to these will often lead to very violent cutaneous disturbances. The reactions vary a good deal, according to the dose given, from a transitory erythema to extensive and often incurable ulceration. Modern appliances are all fitted up with sufficient protective materials against these radiations, and so radio-dermatitis is far less common than what was the case with the earlier workers.

Unlike heat the effect of X-rays is manifested several days (12 to 20) after the exposure to these rays. According to the severity of the lesions, we find erythema, blisters, ulcers, and sloughing. Occasionally the area becomes white or pigmented and indurated, and not infrequently supervention of epithelioma takes place in the chronic forms.

(5) Light

Direct sunlight or any other strong light affects various parts of the body especially the pigment metabolism.

(6) Sound

The constant din in mechanical workshops or noise during war, may produce gross defect in the organ of hearing and may be the main causative factors of neurasthenia.

(7) Chemical Factors

Chemical substances injure the tissues by entering into chemical composition with them and thereby producing a variation in the structure or function of a part or whole of the tissue. Some chemicals act as poison only when introduced in very large quantities, but a considerable quantity of the chemical is excreted or reduced into a harmless form by the activity of the tissues. This is generally effected by various mechanisms. Vomiting eliminates a large quantity of the substances from the stomach in cases of corrosive chemicals. In other instances, a large amount of thick tenacious mucus is formed which mechanically protects the mucous membrane from the irritant action of the poison. A considerable quantity is eliminated after absorption through the kidneys, sweat, bowels and respiration.

General Effects of Injurious Chemical Substances or Poisons

Commonly a poison is taken by the mouth and absorbed through the alimentary tract. In case of snake-bite, the poison is introduced directly into the tissues. The respiratory tract and skin may act as seats for absorption of the poison.

The effect of a poison is either local or general.

Corrosive chemicals, *e.g.*, strong acids, alkalies, or corrosive salts, injure the tissue to a considerable extent and often cause death of the part or they are absorbed into the blood, resulting in general manifestations. When the poison is absorbed, it is carried throughout the whole body and may affect all the tissues, but there is nearly always some particular choice by the poison for some particular tissue. That is the reason why some particular system is more affected than the others in the case of the chemical poisons. Thus, strychnine, narcotics, tetanus toxin have predilection for the central nervous system.

Classification of Poisons

The number of poisonous chemical agents are very numerous. No effort shall be made in this book to give a classification of poisons except the most common forms. The student is referred to textbooks on toxicology for detailed study.

Poisons can be divided into:—

(*a*) Inorganic; (*b*) Organic—(*i*) Vegetable, (*ii*) Animal;
(*c*) Auto-intoxication.

(1) *Inorganic Poisons*

These are commonly the salts of arsenic, mercury, lead, copper and mineral acids.

(2) *Organic Poisons*

Vegetable.

(*a*) These are usually derived from the Natural Orders Apocynaceae, Leguminosae, Loganiaceae, Euphorbiaceae, and Liliaceae. The poisonous elements are derived from the alkaloids, glucosides, alcohols, coal-tar products, etc.

(*b*) The lower vegetable organisms, such as fungus (bacteria and moulds), supply a form of complex substance called toxins, *e.g.*, the toxin of B. botulinus.

(*c*) Food poisoning—Poisonous mushrooms (which contain a highly poisonous alkaloid—muscarin), lathyrism, etc.

Animal.

(*a*) Food poisoning:—

(*i*) Due to the poisonous products normally present in certain animals, *e.g.*, certain poisonous fish.

(*ii*) Due to poisonous products produced after putrefaction of the animals which possess no such poisonous elements when taken fresh. This is exemplified in the case of putrefying fish, milk or meat.

(*b*) Venomous animals:—

Some animals possess glands which secrete highly poisonous chemical substances. These are usually used for offensive purposes as well as for their defence. These chemicals usually belong to the group of proteins such as albumoses. The common animals are the following:—snakes, scorpions, bees, ants, beetles, some species of fish, etc.

(3) *Auto-Intoxication*

This includes the following:—

(*a*) Absorption and retention of poisons produced naturally by the body and prevented from elimination.

Naturally produced poisons are normally eliminated through the kidneys, bowels, etc. Thus any obstructive disorder of these organs will lead to accumulation of these products with toxic manifestations. Disease of the kidneys or obstructive disorder of the urinary passages produces uræmia due to toxic absorption. Similarly, intestinal obstruction or chronic constipation leads to the absorption of fæcal toxin (copræmia).

(*b*) Absorption and retention of poisons produced unnaturally by the body.

These are disorders of metabolism associated with retention of the metabolic products. They may be derived from disturbed metabolism of protein, carbohydrate, fat, purin bodies, etc. The diseases are diabetes, gout, rickets, scurvy, hæmochromatosis, beri-beri, pellagra, etc.

CHAPTER V

INFECTION

Infection is the invasion of the body by living agents with the production of toxic symptoms. The living agents are known as **parasites.**

Parasitism is a condition where a living organism (parasite) exists at the expense of another living organism (host).

The parasites are to be differentiated from **saprophytes** in that the latter live on *dead* organic matter.

Definitions.

A **parasite** is a living agent which obtains its nutrition from another living agent. *Obligatory parasites* are organisms which can live only as parasites and cannot live or multiply outside the host; *e.g.,* gonococcus, malarial parasites. *Optional parasites* are organisms which can maintain their lives as parasites inside the body as well as independently outside the host. *External parasites* or *ecto-parasites* are organisms which live upon the external surface of the host. *Internal parasites* are organisms which live in the natural cavities or tissue fluid of the host. *Facultative parasites* are organisms which ordinarily lead a parasitic life but may live outside the host if necessary. *Facultative saprophytes* ordinarily live outside the host but may also produce disease and become parasites.

A **host** is a living agent which supplies nutriment to the parasite. A *definitive host* is the animal in which the parasite exists during its sexual life; *e.g.,* mosquito and malarial parasite. An *intermediate host* is the animal in which the parasite lives during its asexual life; *e.g.,* man and the malarial parasite. **Mixed infection** is that in which more than one parasite infect the organ simultaneously. **Secondary infection** is that in which parasites (secondary) invade the host after or along with the production of a disease in the same host by some other parasites (primary).

Exogenous infection is that which is due to the entry of the organisms from without. **Endogenous infection** is that which is due to organism existing within the individual as simple parasites but causing infection due to a temporary lowering of the resistance of the host. **Focal infection** is one which acts as the initial point of infection (a focus) for dissemination of the infectious material to other parts of the body.

Toxæmia—Septicæmia—Pyæmia.

* **Toxæmia** is a condition in which toxins elaborated at some local site of infection are absorbed into the tissues and then into the circulation; *e.g.*, in diphtheria, tetanus, erysipelas.

Septicæmia is the condition in which the infective micro-organisms invade the blood stream and multiply therein, *e.g.*, pneumonia, typhoid, streptococcus, staphylococcus (in puerperal fever, post-mortem or post-operative wounds).

Pyæmia is the condition in which the septicæmia is complicated by multiple abscess formation in different parts of the body. It must be noted that all septicæmic cases are not pyæmic but all pyæmic ones are septicæmic. According to anatomical peculiarity, pyæmia may be divided under three heads: (*i*) arterial pyæmia, *e.g.*, in infective endocarditis, (*ii*) systemic venous pyæmia, *e.g.*, in osteomyelitis, cellulitis, etc., (*iii*) portal pyæmia, *e.g.*, in suppurative pylephlebitis or from infection from the alimentary tracts or gall bladder.

Route of Infection.

The following are the gates through which the pathogenic organisms enter into the human organs:—

1. **Through the skin.**—Normally the skin serves the purpose of protection against the invasion of organisms. But, on account of its exposed surface, it is liable to injuries, and not only the visible wounds but also the minute abrasions serve as gateways for the entrance of bacteria. The organisms may enter through the openings of the sweat-glands without any lesion of the skin. Furuncles can be experimentally produced by rubbing a pure culture of staphylococcus aureus on uninjured skin. Many animal parasites, *e.g.*, those of malaria, filaria, etc., are introduced through the skin by the bite of mosquito or other carriers.

2. **Through the alimentary canal.**—The alimentary tract is normally full of bacteria, mainly B. coli, but often one finds infection through this tract. In the mouth, the tonsils are often found to be the primary portal for the invasion of tubercle bacillus, staphylococci, etc., nasopharynx for that of meningococcus, diphtheria, poliomyelitis virus, etc. The mouth is also the seat of thrush.

Stomach, on account of its acid secretion, is somewhat protected, but that protection is not absolute. Bacteria and many animal parasites pass uninjured through the stomach and cause diseases, *e.g.*, cholera.

3. **Through the respiratory tract.**—The organisms are generally taken in by inhalation and then infect the respiratory tract, *e.g.*, pneumonia, tuberculosis, diphtheria, influenza, etc.

4. **Through the urogenital tract.**—This forms the portal for venereal diseases (syphilis, gonorrhœa, etc.). In women, B. coli and streptococci often enter through the genital tract.

5. **Through the blood direct.**—This often takes place when the organisms, specially of some tropical diseases, are directly introduced into the blood by the bite of the insect. Septicæmia often occurs with cocci, without any demonstrable primary lesion.

Adaptation.

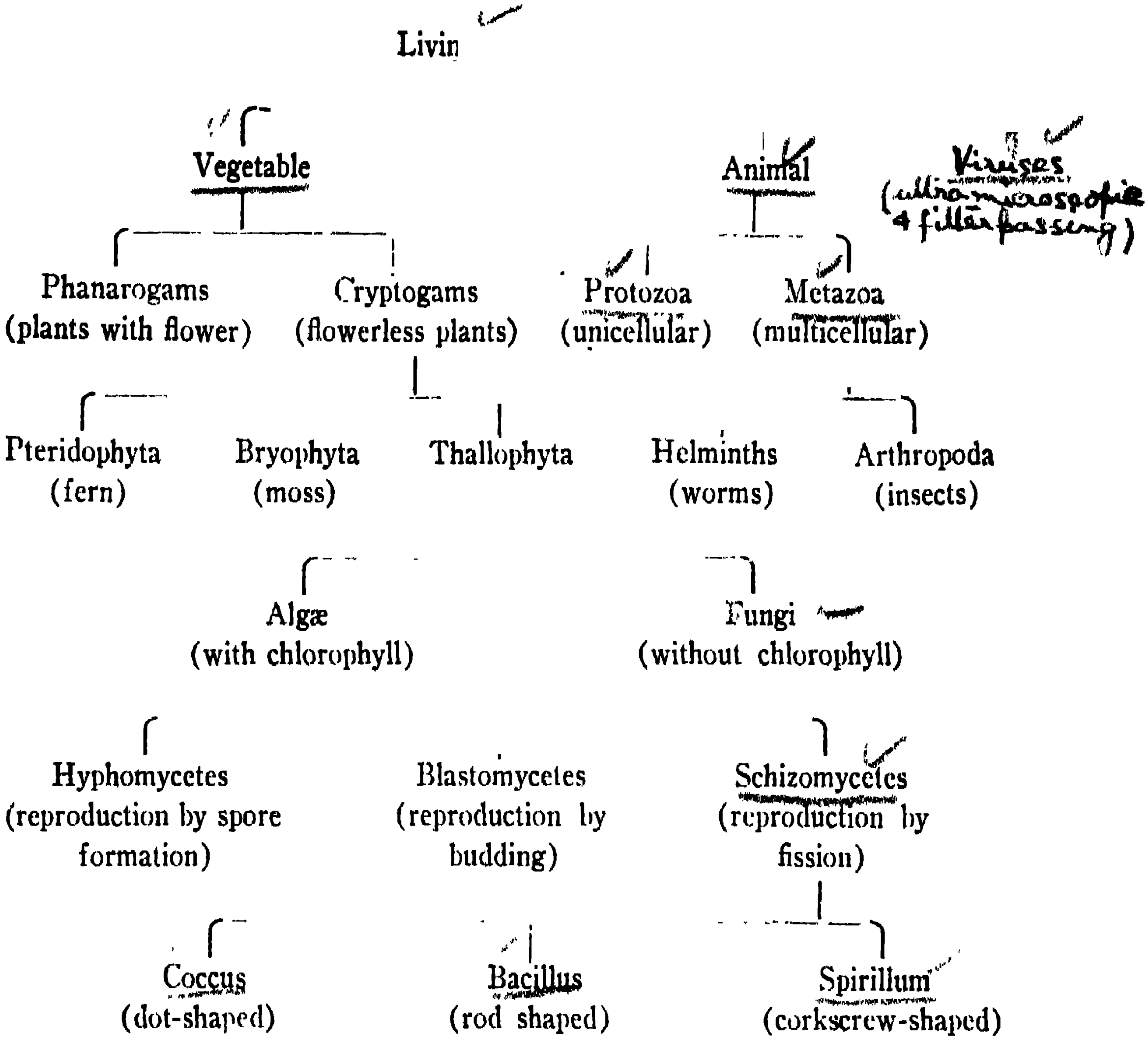
The feeding apparatus of the parasites, especially those of the higher animals, are adapted according to the nature of the food material they have got to take. For example, the mouth parts of the blood-sucking insects are suitable for both piercing and sucking, whereas those of the common house-fly are suitable only for sucking.

The insects usually transfer themselves from one host to the other but this is impossible for the internal parasites to do. Thus, the tape worms, on account of their existence as obligatory parasites, have lost their circulatory, respiratory, alimentary and even sense organs, and so are absolutely incapable of adapting themselves to any new environment. In other instances we find that special adaptations are provided for the progeny of a parasite when the adult one cannot possibly transfer itself to a new host.

Parasites may be divided into 3 ~~two~~ great divisions:

(1) vegetable ~~and~~ (2) animal. + 3 Viruses

The relation of these parasites to one another is shown in the following table:—



Of the vegetable organisms, the study of hyphomycetes and blastomycetes comprise the science of **Mycology**; and that of Schizomycetes comprise the science of **Bacteriology**.

Of the animal organisms, the study of protozoa comprise the science of **Protozoology**; that of helminths comprise the science of **Helminthology**; and that of arthropoda comprise the science of **Entomology**.

CHAPTER VI

FUNGI—MOULDS

Hyphomycetes

These are fungi consisting of long filamentous tubular branches which form a mycelial mass. They are multicellular and send out upright processes, known as **hyphæ,** at the end of which special reproductive elements are produced. These reproductive elements are the **spores.** The reproductive elements are present only in some specialised cells and so the cells are interdependent. The genera, important to human pathology, are subdivided into two main groups:—

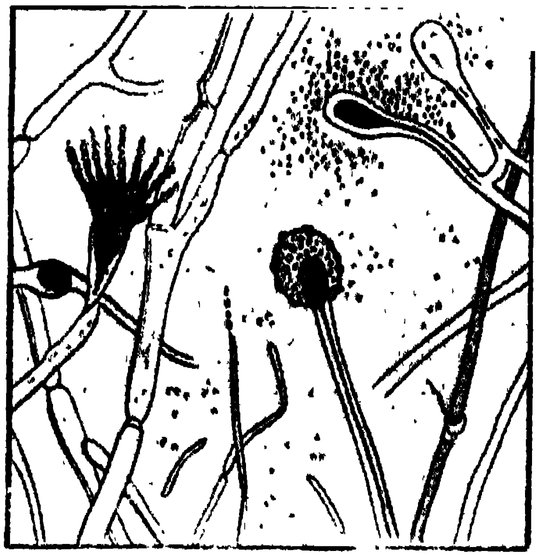


Fig. 19. Showing general characters of Hyphomycetes.

(*a*) Those without branches are called **leptothrix** and

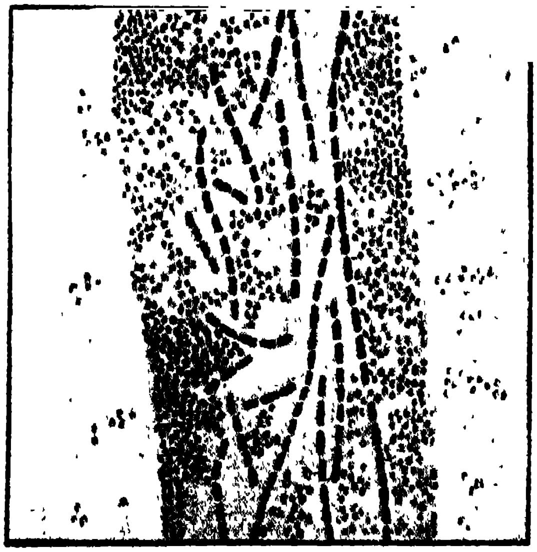


Fig. 20. Ringworm affecting hair.

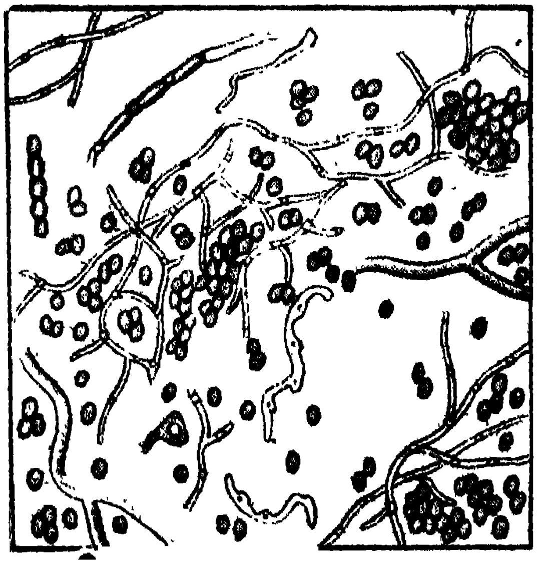


Fig. 21. Favus from scrapings.

(*b*) Those, showing true branchings, with the filaments interlacing one another, forming what is known as mycelium, are called **streptothrix.**

They are commonly responsible for the different forms of the skin diseases of man. The so-called eczema-like conditions of man, in this country, are very often due to a species of ringworm fungus.

1. *Trichophyton tonsurans* or the Ringworm fungus (large spored).—This organism usually grows upon the skin and the nails. It enters into the skin and grows between the horny and the cellular layers of the epidermis. The hair is often attacked and the organism enters into the shaft of the hair thus making it brittle. The hair follicles are also affected sometimes. The spores measure 5—8μ in diameter.

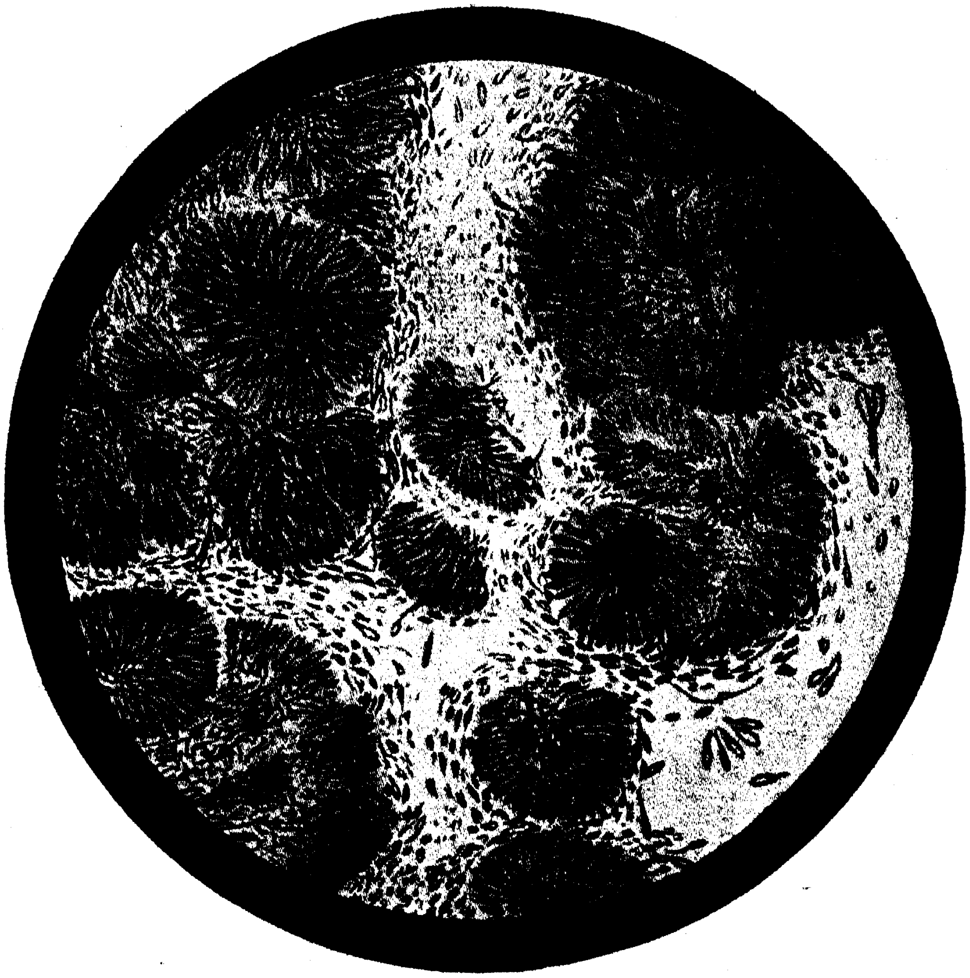


Fig. 22. Actinomycosis—washed isolated grains are mounted in 50% glycerine on a slide with a cover glass and on applying steady and gentle pressure the grains are flattened out. (From Dr. Taraknath Sur).

2. *Microsporon audouini* or the Ringworm fungus (small spored). The spores measure about 2 μ in diameter.

Microspora produce similar lesions to those produced by Trichophyta.

3. *Microsporon furfur* or tinea versicolor or pityriasis versicolor or the fungus causing pityriasis. This organism affects the skin, especially of the trunk and the limbs, and produces pale yellowish or whitish yellow discoloration. It is believed that this is due to the saprophytic growth of the organism on the surface of the skin and is not a disease in itself.

4. *Microsporon minutissimum.*—This is the fungus causing the affection of the skin known as erythrasma.

5. *Oidium albicans.*—This affects the mucous membrane of the tongue, mouth, palate, stomach, vagina etc., with the formation of white patches causing the disease known as thrush or soor. This is generally observed in children suffering from wasting diseases or marasmatic conditions. Monilia, a form of oidium, is said to cause sprue.

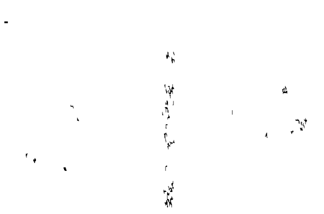


Fig. 23. Colonies under magnifying lens showing central elevations, depressions and fringed margins at the base.

General Characters of these Organisms.

These organisms are found in the form of fine mycelial threads, with occasional formation of spores. Each mycelium is composed of numerous branched filaments, called hyphæ.

Microspora are known as the small-spored ringworm parasites, whereas the Trichophyta are the large-spored ones. The mycelium of these organisms penetrates into the hair and grows inside them and proceeds upwards and downwards along the hair both inside as well as on the surface.

Demonstration of the Fungus.

The hairs or the scales are taken on a slide on which a drop of liq. potassæ (B.P.) is put. It is covered with a coverglass and gently heated over the burner for a few seconds, and then examined under the microscope. The hairs and epidermis are cleared by the alkali, rendering the fungus easily detectable.

Staining.

Remove fat from the hair or the scale by chloroform. Put the specimen in warm formic acid. Wash acid with water and stain with Sahli's blue [*Methylene blue, saturated aqueous solution—3 parts, solution of borax* (5 %)—*2 parts, aqua distil.—5 parts*]; wash, then dehydrate in absolute alcohol, clear in xylol and mount in canada balsam.

These organisms are Gram-positive.

Cultivation.

All of these organisms can be cultivated without any difficulty. The best medium for their cultivation is Sabouraud's medium,* in which the fungi have characteristic growth, and, at the same time, the growth of ordinary skin organisms, especially the cocci, is inhibited. The colonies are usually white, but vary from greyish to yellowish white in colour. The colonies may appear as thin layers, or may be heaped up in the centre and much raised above the surface. The surface may be moist, shining or dry, wrinkled, and woolly.

The morphological appearances vary occasionally according to the chemical constituents of the culture media. They also grow well upon the ordinary media, *e.g.*, agar-agar, broth, blood serum, milk, egg, etc.

6. *Achorion Schönleinii* or tinea favosa or favus fungus.—This organism is the cause of favus, a disease usually occurring upon the head affecting the hair. It may affect other parts of the body—even hairless portions. The characteristic appearance of this lesion is a cup-shaped depression of the skin, known as the "favus-cups." They are covered with a yellow crust, through which the lustreless, brittle and loose hairs project. The crusts consist of large number of hyphæ and mycelial filaments.

7. *Aspergillus.*—There are three forms of this fungus, differentiated by the colour of the mycelial masses:—

(*a*) Aspergillus flavus—yellow in form. Not of much importance.

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*Sabouraud's medium consists of *Peptone*—1 per cent., *Maltose*—4 per cent., *Agar*—1·8 per cent., made up as in cases of ordinary agar.

formation of multiple sinuses, with occasional discharge of small granular bodies. The organism never produces any secondary lesion in the internal organs.

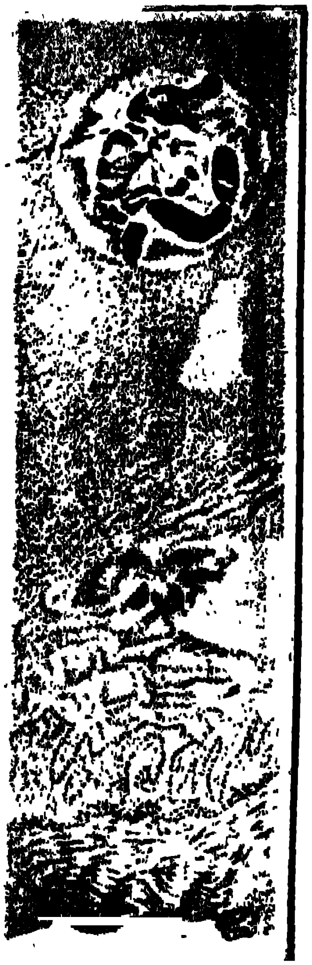

Fig. 25. Section of a piece of tissue removed from the growth shown in Fig. 24. Stained by Haematoxylin. Magnification 350. The fungoid element, black in color, is seen in the depth of tissue.
(From
Dr. Gopal Ch. Chatterjee.)

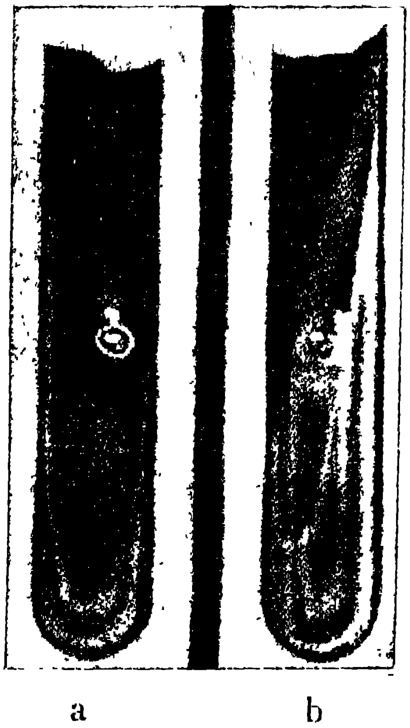

Fig. 26. Shows the appearance assumed by the granules removed from the affected tissue when placed in agar after 4 days incubation at 37°C. The figure (a) shows the front view of the growth and (b) the side view. The growth is raised from the surface of the agar and is spherical in shape. There is no tendency to spread. The radiating hairy structures can be seen growing from the colonies. The agar tinged a little black around the growth.

(From Dr. Gopal Ch. Chatterjee.)

There are three forms of mycetoma according to the colour of these granules—white, black, and red—the last variety being the rarest. The white and red varieties belong to the group of streptothrix, whereas the black one belongs to the group of hyphomycetes. The red variety is very rare.

Microscopically, these grains present only masses of branched mycelial filaments, with the absence of clubs which characterise the actinomyces bovis. The organism stains well with ordinary stains, as well as, by Gram's method. They can be cultivated easily in the usual laboratory media.

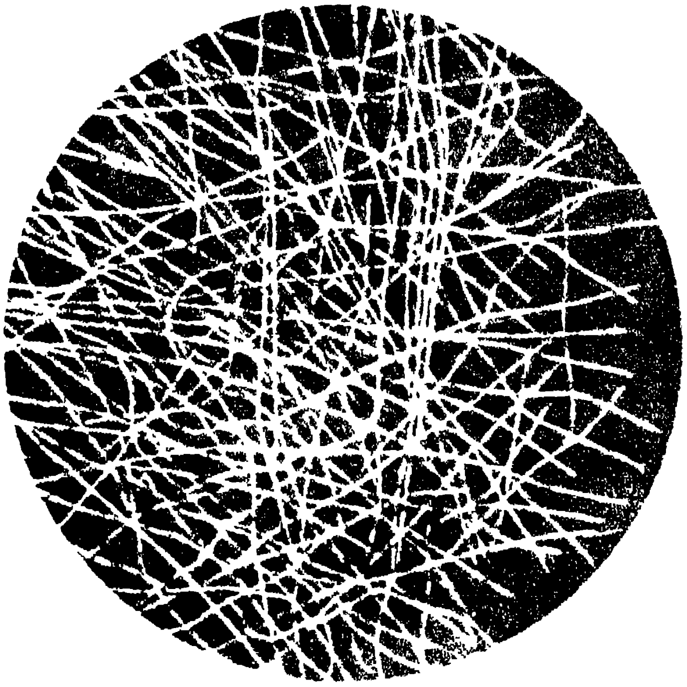

Fig. 27. A portion of a colony removed from the wall of broth culture of black Mycetoma, stained by iron-hæmatoxylin, showing the mycelium.
(From Dr. Gopal Ch. Chatterjee.)

11. *Epidermophyton inguinale.*—This organism causes eczema marginatum, and is closely allied to the group trichophyta.

12. *Sporotrichon.*—This fungus produces granulomatous lesions of the skin and other parts of the body, often simulating those of syphilitic or tuberculous origin. The condition is called sporotrichosis. They often produce abscess, and in fact, it was originally described from a case of chronic subcutaneous abscess in America.

The organisms are usually found in the pus, or inside the granulation tissue, in the form of spores, 3-4 μ long, and, very rarely, in the form of mycelial threads.

They readily grow on culture media, with formation of raised, white or white-brown fluffy colonies, in which the formation of mycelial threads are abundant.

Blastomycetes

These are also known as *saccharomycetes* (on account of the property of fermenting sugar), yeasts or budding fungus (on account of the reproduction by budding). They are of very great importance to the chemist and the brewer.

(These are unicellular, small ovoid organisms, measuring 8 to 9 μ, in diameter, which multiply by gemmation or budding. Buds appear from either the sides, or from one or both ends, and increase gradually in size. When these buds reach a considerable size, they are detached from the mother cell, and become separate individuals.

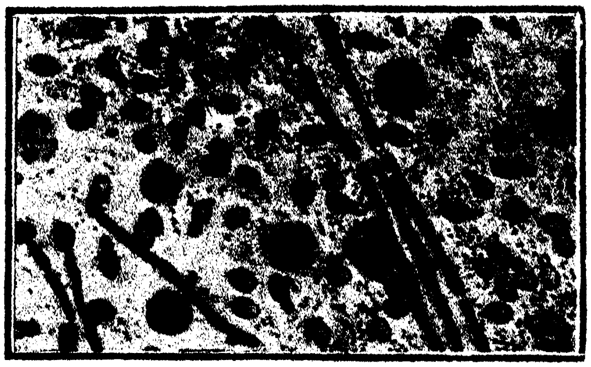

Fig. 28. Budding form of yeast-like cells with mycelial threads from sputum of a case of Broncho-moniliasis.
(From Dr. Taraknath Sur.)

The ovoid cell consists of a double-contoured cell wall of cellulose, and a granular protoplasm containing drops of fat or vacuoles. No nucleus is visible. In old cultures, involution forms are often met with. They stain readily with aniline dyes, and, also, by Gram's method. They can be easily cultivated on ordinary media, preferably, Sabouraud's medium.)

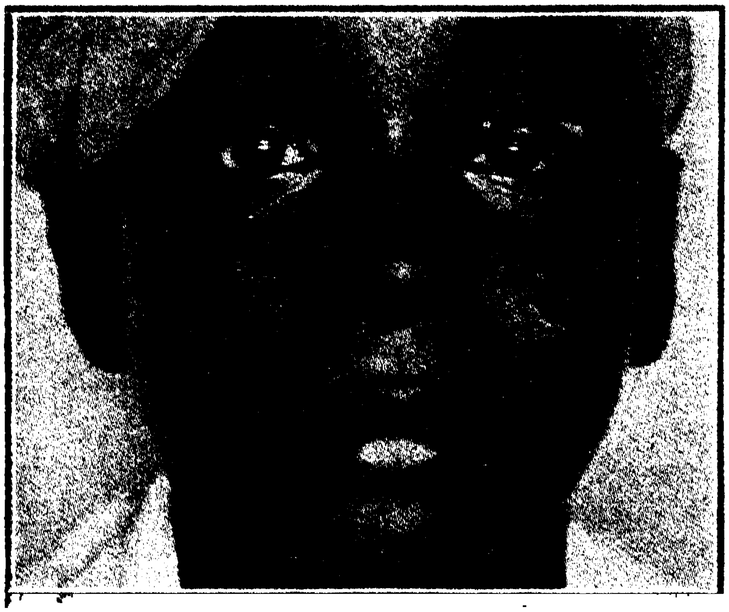

Fig. 29. Blastomycosis. (From Dr. Taraknath Sur.)

Apart from the multiplication by budding, spore formation has been observed, and tendency to form mycelia in culture is frequently observed also.

The lesion caused by the organism is a form of skin affection, known as *blastomycotic dermatitis.* This is characterised by the presence of small miliary abscesses in the *rete* and the corium, the pus of which contain the yeast cells. Sprue has often been attributed to yeasts, but this has not yet been definitely proved.

CHAPTER VII

SCHIZOMYCETES or BACTERIA

Bacteria are a form of low unicellular vegetable organism, devoid of chlorophyll. The protoplasm is homogeneous, there is no nucleus,* and multiplication takes place by transverse division or fission. They often collect in masses embedded in glutinous envelope, and are known as zooglœa masses.

Part Played by Bacteria in Nature

Bacteria are the main agents in the processes of putrefaction, fermentation, digestion, nitrification, and disease production.

Putrefaction.

This is the process in nature's economy, by means of which the dead bodies of vegetable and animal organisms are disposed of by the formation of substances useful for the growth of other vegetable and animal life.

Without bacterial aid, all these dead substances would have remained sterile, and there would have been no change and utilization of the organic chemicals. Thus, by the process of putrefaction, bacteria act as scavengers by removing the dead remains of vegetable and animal organisms, and converting them into simpler organic compounds, which act as manures to foster the growth of higher vegetable and animal life.

Fermentation.

Micro-organisms are the agents for producing a great variety of ferments under different conditions. For example, milk is fermented by the organism lactic acid bacillus. Similarly, other acids are produced as products of fermentation notably the formation of acetic acid from fermentation of vinegar. Urine is fermented by micrococcus ureæ, with the production of ammonia. It is a curious feature to note that, in cases of fermentation, the process proceeds up to a certain stage, after which it stops spontaneously by the inhibitory action of the product of fermentation on the organisms themselves.

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* Morphologically no nucleus can be demonstrated, and if there be any nuclear substance in the protoplasm, it must be diffused throughout the cytoplasm.

Digestion.

The process of digestion is carried normally by the ferments and enzymes present in the alimentary tract, each of them having properties of digesting some particular proximate principles constituting the diet. In addition to these ferments and enzymes, micro-organisms present in the alimentary tract, particularly the bacillus coli, help a good deal in digesting the elements.

Nitrification.

Micro-organisms present in the soil play a great part by converting the unstable nitrogenous substances into stable forms, the micro-organisms chiefly concerned being the nitrobacta and nitrosomonus.

Nitrogenous substances undergo decomposition, resulting in the formation of ammonia. Now, by the action of the nitrifying organisms present in the soil, ammonia is changed into nitrous acid which, combining with the bases, forms *nitrite* (the result of incomplete nitrification—the bacteria concerned being chiefly nitrobacta). This is again oxidised into nitric acid, which combines with the bases to form *nitrate, i.e.,* the stable form of nitrogenous split products.

Sometimes, owing to the action of microbes or reducing agents, nitrate is deoxidised into nitrite, and this again into ammonia.

Disease Production.

A fairly large number of bacteria cause diseases and are hence termed pathogenic micro-organisms. Apart from pathogenesis, bacteria may also cause fermentation, putrefaction, digestion, etc., and, in some instances, the pathogenic effect is actually due to these products. Thus, in the process of putrefaction of proteins, some substances, such as ptomains, are produced, which are highly poisonous to human beings, causing violent vomiting, purging, etc. This condition may be due to the ingestion of ptomain bodies, or to the production of similar substances in the intestines by the organisms.

Diseases are mainly caused either by the organisms themselves, or by their products.

Products of Bacteria

These are mainly:—

- (*A*) Pathogenic.
- (*B*) Putrefactive.
- (*C*) Fermentative.
- (*D*) Enzymes.
- (*E*) Chromogenic.
- (*F*) Bacteriophage.

(A) Pathogenic.

These usually occur in the form of toxic substances produced by the growth of the organisms. They are of the following classes—

(1) *Endotoxin or intracellular toxin.*—These are toxins produced by the organisms and kept inside the body of the cells, and are not excreted into the surrounding culture medium unless they are disintegrated. Thus, if the liquid medium used for the cultivation of the organisms, *e.g.*, organisms of the typho-coli group, be filtered, so as to separate these organisms, the filtrate contains very little or no toxin at all, unless the growth contains the disintegrated bodies of the organisms. On the other hand, if the bodies of the organisms be broken up by grinding, and emulsified with saline, the latter is found to be toxic. Most of the common organisms produce

(2) *Exotoxin or extracellular toxin.*—These products are found during the growth of the organisms, and are rapidly excreted in the culture medium. They are soluble in the liquid medium (broth) used for the cultivation of the organisms, the best example being B. tetani. If the liquid culture medium after the growth of B. tetani be filtered, so as to separate the fluid from the bodies of bacteria, the fluid is found to be exceedingly toxic. It will kill an animal weighing a million times the weight of the toxin. It is destroyed by prolonged heat at 60°C, and can be oxidised into a harmless product. *

(3) *Toxic substances produced by the action of organisms on the protein substances present in the culture medium.*—These are either due to digestive or destructive action on the proteids. The products are mainly albumoses in nature. During their invasion into the human tissue, the proteids of the tissue are similarly converted into poisonous substances, and produce the diseased condition. In some instances, the proteids are, by further metabolic changes, reduced to non-proteid alkaloidal substances, which are even more toxic than the albumoses. The albumoses

produce fever and fatty degeneration, especially of the heart muscles The alkaloids produce local œdema and vesicles which are most commonly found in case of infection with B. anthracis. Injection of various killed bacterial cultures often produces suppurative areas due to the chemotactic influence of the bacterial protein (the so-called mycoproteins). The bacterial protein can also be used as foreign protein to induce anaphylactic phenomenon.

(4) *Toxones.*—These are toxic substances, as may be found in the culture of diphtheria bacillus along with the exotoxins; but they differ from these, in being less toxic, and the effect is not so acute. They produce antitoxin, but possess feeble affinity for it.

(5) *Toxoids.*—These are non-toxic substances produced by the organisms, *e.g.*, B. diphtheria and tetani; but they combine with antitoxins, and produce antitoxins when injected into animals. These indicate the presence of only the haptophore group of the toxin, and not the toxophore one.

(6) *Hæmolysins.*—These are substances which lead to the laking of red blood cells. This phenomenon of hæmolysis is often used to differentiate pathogenic from non-pathogenic bacteria.

(7) *Leukocidins.*—These are substances which lead to solution of leukocytes.

(8) *Aggresins.*—*See* Chapter on Immunity.

(B) Putrefactive or Decomposition Products.

These are products derived from the decomposition of organic, mainly protein, matters after death of the animal or vegetable.

The products are, proteoses, peptones, leucin, tyrosin, indol, skatol. phenol, volatile acids, sulphuretted hydrogen, methane, ammonia, carbonic acid, hydrogen, etc.

(C) Fermentative Products.

These are mainly derived from the fermentation of sugars. They are, alcohol, carbonic acid, lactic acid, butyric acid, acetic acid, other acids, etc.

(D) Enzymes.

The bacteria secrete substances called enzymes, the activities of which produce the changes taking place in putrefaction and fermentation. These are regarded to be a special kind of protein which acts as a catalytic agent, *i.e.*, it takes part in the reactions but is not used up in the process. These enzymes, like toxins, are both extracellular and intracellular. The former form is found free in the culture media, whereas the intracellular form is present inside the cell. The enzymes are divided into—

(1) *Hydrolases.*—These bring about the changes by the addition of water, *e.g.*, in the inversion of cane sugar by invertase.

(2) *Oxidases.*—These bring about the changes by the addition of oxygen to the molecule, *e.g.*, oxidation of ethyl alcohol to acetic acid by bacterium aceticum.

(3) *Zymases.*—These bring about the changes not by hydrolysis or oxidation but apparently only by splitting up the compounds into simpler ones. This is produced by yeast cells, etc. Zymases convert glucose into alcohol and carbon dioxide.

(E) Chromogenic Products.

Some organisms produce pigment when grown on artificial medium, hence the term *chromogenic bacteria* is applied to them. The common examples are B. pyocyaneus and B. prodigiosus which produce blue and red pigment, respectively. This pigment is insoluble in water but soluble in alcohol, ether or carbon bisulphide.

In water grows a species of bacteria (bacillus fluorescence liquefacieus) which produces a green or greenish-yellow pigment. This pigment is soluble in water and so colours the surrounding medium, but is insoluble in alcohol, ether and carbon bisulphide. This appears yellow by transmitted light and green by reflected light. These are called *fluorescent bacteria.*

Several species of bacteria occur in sea water which produce substances which are luminescent in the dark. These were formerly termed as *phosphorescent* but are properly termed as *luminescent.*

(F) Bacteriophage.

Historical.—It was in 1896 when Hankin observed that the number of organisms varied a good deal in natural conditions in the river Jumna. Just below Agra, it was more than 100,000 organisms per c.cm. while only 3 miles further down 1 c. cm. of the river water contained less than 100 organisms. He further demonstrated that cholera vibrio were destroyed in the test tube when kept in the filtered river water. This property of lethal action was destroyed by boiling which led

Hankin to suppose that this property was due to the presence of some volatile substance in the water of the Jumna.

In 1915 Twort observed that the colonies of cocci isolated from vaccine lymph became transparent and glossy, with disappearance of the coccal form and showing unrecognisable debris. This glossy degeneration could be transmitted through an indefinite generation and could pass through Pasteur-Chamberland filters.

In 1917 d'Herelle made the monumental discovery of the phenomenon which is known as the *bacteriophagy.*

Experimental.—D'Herelle first observed that when a few drops of a bacteria-free filtrate from the stools of a dysentery (Shiga) patient was added to a young broth culture of *B. dysenteriæ* (*Shiga*) and incubated, the turbid broth culture instead of becoming more turbid, as expected, became less and less turbid and finally became completely clear in 12 to 24 hours. The bacilli have completely disappeared and were no longer to be seen on microscopical examinations. Subcultures from this clear broth culture remained sterile.

He further found that a small drop of this clear culture when added to another young broth culture of Shiga bacillus shows the same phenomenon of complete lysis within a short time. Inoculation of the third culture with a drop from the second only repeats the process. This proved that the lytic principle can be transmitted *ad infinitum* without any loss of its substances. D'Herelle further observed the same phenomenon on solid medium with surface cultures. He mixed a very small quantity of the filtrate from the dysentery stool or a drop from the lysed culture to a young broth culture of Shiga bacillus and made subcultures from this mixture upon agar at regular intervals. After inoculation the surface growth of the organisms showed sharply-outlined circular zones of clear areas showing no growth at all and also showing absence of organisms. Each clear zone represents a colony of bacteriophage corpuscle and that derived from a single corpuscle. Since d'Herelle's original observations these experiments have been repeated hundreds of times not only by d'Herelle himself but by numerous other workers too and are now free from any dispute.

Source of Bacteriophage.—It was originally isolated from fæces of a dysentery patient. Since then it has been repeatedly isolated from fæces in nearly all convalescent cases of dysentery as well as from cholera and other intestinal affections. It has also been isolated from fæces of healthy human beings as well as that of animals. It has been found in water, sewage, pus, exudates etc.

General Characters of the Bacteriophage.—The lytic principle is ordinarily specific but occasionally shows evidence of non-specificity. Thus a given "*phage*" may have a powerful action upon Shiga bacillus, a moderate action on B. paratyphosus B and a weak one on B. coli. The activity of the "phage" has been extensively studied with organisms belonging to the coli-typhoid-dysentery group. Other organisms *e.g.*, staphylococcus, B. pyocyaneus etc., also show the phenomenon of bacteriophagy. The general characters of bacteriophage lysis may be summarised as follows.—

> It produces partial or complete lysis of young broth culture of the organisms. It is most active against the particular sensitive bacteria, less so against closely related species, while inactive against other organisms. The reaction is in most instances specific. It produces a "bitten" or "glassy beaded" appearance on solid cultures. The lytic principle can pass through Pasteur-Chamberland filters, its diameter being about 20 $\mu\mu$[*]. It resists a temperature of 56-58°C, but is destroyed at 75°C. It can grow both ærobically and anærobically and can be cultivated for generations serially for an indefinite period, but only when it is grown in association with living sensitive organisms but not when grown on bacteria-free medium or with dead bacteria. It is active in such a high dilution as a ten-billionth part of a cubic centimetre (10^{-10} c.cm.). The bacteriophage is widely distributed and is always found in association with living bacteria. The sensitive bacteria can be trained to be resistant to the lytic action of the corresponding phage. Bacteriophage has got the property to behave as an antigen. Rabbits can be immunised against the phage and antilytic serum can thus be prepared.

Secondary Cultures.—With bacteriophagy it seems that all the bacteria present in the suspension have been destroyed, but very often it is found that some of the organisms survive even after apparent complete destruction. This is seen both in liquid as well as on solid media. In liquid media the suspension becomes perfectly clear within a fairly short time, but on further incubation the media often become turbid again and subcultures show growth of the original organism.

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[*] $\mu\mu$ (millicron) is the thousandth part of μ (micron), which latter, again, is a thousandth part of one millimeter.

Upon solid media the bacterial growth becomes clear but after a certain period small tiny colonies appear in the clear area. These growths are termed "secondary cultures" by d'Herelle.

The secondary cultures show marked differences from the original parent strain. Thus agglutinating parent strains often show only partial or no agglutination in secondary culture; the morphological appearance changes and loss of motility takes place.

The Nature of Bacteriophage.—This remains still a disputed point to settle, and we are still far from the final conclusion. The main theories are:—(1) Theory of d'Herelle.—From the property of serial transmission *ad infinitum,* d'Herelle supposes this to be a living virus and on account of its size he calls this an ultramicroscopic filter-passing living micro-organism, which multiply in the culture of bacteria of which the "phage" is the obligatory parasite. From the presence of clear areas in solid culture called by d'Herelle as "colonies of the phage," it is evident that the lytic principle is a minute particulate body for which again, d'Herelle coins the term *protobe.* (2) The other schools believe this lysis to be due to the action of autolytic ferments. It is supposed that the dissolution of the bacteria leads to the freeing of the lytic substance which starts the lytic process and continues in series *ad infinitum.* The main objection to the theory is the difficulty to account for the origination of the process. Further, autolysis of the bacteria is observed in old cultures only, while the bacteriophage is found to be most active in fresh cultures. (3) Bordet believes that due to some mutational changes an actively lytic substance is set free which is the cause of autolysis. (4) Twort regards the d'Herelle phenomenon identical to that discovered by himself in 1915 but d'Herelle regards this a quite different phenomenon on the ground that unlike Twort in d'Herelle phenomenon no residue is left in the form of debris; the destruction of the organisms is complete and absolute.

Bacteriophage and Infections.—D'Herelle supposes that natural cure of infectious diseases mainly takes place by the action of the phage. At the commencement of the disease, as no phage is present, the disease advances until phage production takes place. When the phage is of sufficient "virulence" the organisms are destroyed and cure takes place. This applies not only to isolated cases of infectious disease but also explains natural cessation of epidemics. D'Herelle conceives "the beginning of an epidemic is marked by the diffusion of the causal bacteria; its end by the diffusion of the bacteriophage, virulent for this bacterium."

Classification of Bacteria

Classification of Bacteria.—This depends mainly on the physiological and biological characters of the organisms as also on the morphology and pathogenicity of the same. Classification on purely morphological characters is undoubtedly a very simple one but to the modern bacteriologist convey a very meagre impression. As for instance, on account of the shape being like a rod, one organism is termed as a bacillus and one may cite some examples in the bacillus tuberculosis, bacillus anthracis, bacillus typhosus, bacillus pestis etc. The modern bacteriologist will call these organism by the terms—Mycobacterium tuberculosis, Bacillus anthracis, Eberthella typhi, Pasturella pestis respectively. The importance and utility of this classification is that, if the general characters of the genus are known it will at once bring to the reader the idea of the organism in question such as, Mycobacterium smegmatis, Bacillus subtilis, Eberthella dysenteriæ (shiga), Pasturella aviseptica etc.

The following chart is based on the modern classification (of the Society of American Bacteriologists). The bacteria are usually classified under Order, Family, Tribe, Genus and Species.

ORDER.	FAMILY.	TRIBE.	GENUS.	SPECIES.
Actinomycetales	Actinomycetaceæ		Actinomyces	A. bovis, A. maduræ.
	Mycobacteriaceæ		Mycobacterium	M. tuberculosis, M. smegmatis.
			Corynebacterium	C. diphtheriæ, C. pseudodiphthericum.
			Fusiformis	F. dentium (in Vincent's angina).
			Pfeifferella	P. mallei.
Eubacteriales	Nitrobacteriaceæ		(Mainly soil bacteria)	
	Spirillaceæ		Vibrio	V. comma, V. metchnikovi.
			Spirillum	S. undula (found in water or putrid infusions).
	Coccaceæ	Neisserieæ	Neisseria	N. gonorrhœæ, N. intracellularis. N. catarrhalis.
			Gaffkya	G. Tetragena.
		Streptococceæ	Streptococcus	S. pyogenes, S. hæmolyticus.
			Diplococcus	D. pneumoniæ.

ORDER	FAMILY	TRIBE.	GENUS.	SPECIES.
	Coccaceæ (*Contd.*)	Micrococcaceæ	Staphylococcus	S. aureus.
			Micrococcus	M. ureæ, M. luteus (usually non-pathogenic).
			Sarcina	S. ventriculi.
		Chromobactereæ	Chromobacterium	C. violaceum.
			Pseudomonas	P. pyocyaneus.
Eubacteriales (*Contd.*)	Bacteriaceæ	Bacterieæ	Proteus	P. vulgaris.
			Escherichia	E. coli, E. acidi lactici.
			Ærobacter	A. ærogenes (lactis ærogenes).
			Salmonella	S. paratyphi (para A), S. schottmulleri (para B).
			Eberthela	E. typhi (typhosus), E. dysenteriæ (shiga). E. paradysenteriæ (flexner, Y).
			Alkaligenes	A. fæcalis.
		Pasteurelleæ	Pasteurella	P. pestis.
		Hæmophileæ	Hæmophilus	H. influenzæ, H. pertussis, H. ducreyii.
			Brucella	B. melitensis.
		Klebsielleæ	Klebsiella	K. pneumoniæ (pneumobacillus).
		Bacillaceæ	Bacillus	B. anthracis, B. subtilis.
			Clostridium	C. tetani, C. welchii, C. œdematis maligni (vibrion septique).
Spirochætales		Spirochætaceæ	Treponema	T. pallidum.
			Borrelia	B. recurrentis, B. vincenti.
			Leptospira	L. icterohæmorrhagiæ.
			Cristispira	C. balbiani.
			Spirochæta	S. plicatilis.
			Saprospira	S. grandis.

For the purpose of convenience, the following classification may be adopted, as it is very helpful for the students to remember the names, staining, and cultural characters of the different bacteria:—

(A) Cocci.

Gram-positive Staphylococcus, Streptococcus, Pneumococcus, M. tetragena.

Neissaria group e.g. –

Gram-negative Gonococcus, Meningococcus, Micrococcus catarrhalis, M. melitensis. G. tetragena -
B

(B) Bacilli.

1. **Acid-fast Group.** (Mycobacteria).
   B. tuberculosis, B. lepra, B. smegma, Butter bacillus.

G positive

2. **Spore-forming Group.** (Myc [illegible]
   (a) *Ærobic.*—B. anthracis, B. Mycoides, B. subtilis.
   (b) *Anærobic.*—B. tetani, B. ærogenes capsulatus, or welchii
   B. œdematis maligni.—B. botulinus, B. enteritidis sporogen, B. of symptomatic anthrax, B. butyricus.

3. **Diphtheria Group.**
   B. diphtheria, B. hofmann, B. xerosis.

Gra. negative

4. **Hæmophilic** (requiring blood in the culture medium).
   B. influenza, B. pestis, B. pertussis, B. koch-weeks B. ducrey, Pneumobacillus of Friedländer, Morax-Axenfeld diplobacillus.
5. **Intestinal.**
   B. coli, B. typhosus, B. para-typhosus A & B B. shiga, B. flexner, B. gaertner, B. proteus.
6. **Chromogenic** (pigment producing). B. pyocyaneus, B. prodigiosus.

(C) Spirilla.

Gran egative.

1. **Vibrio.**
   Vibrio choleræ.

. **Spirochætes.**
   T. pallida, T. pertenuis, S. recurrentis, Leptospira icterohæmorrhagæ, L. icteroides, S. vincenti, S. refringens.

Effect of Various Conditions on the Growth and Life of Bacteria

1. Temperature.

In order to be pathogenic, it becomes necessary that the organisms should grow with full activity in the body temperature of the human being. They grow best (optimum temperature) at blood heat (37.5°C). They can live also at other temperatures although they are not able to multiply. The temperatures above and below which no growth will take place, are called the maximum and minimum temperatures respectively. Thus, freezing does not generally kill the organisms (cholera vibrio, typhoid bacilli, etc.,) but their growth is suspended, and they become active again and multiply readily as soon as the temperature is raised. Typhoid bacilli can withstand the lowest temperature available (—300°C of liquid hydrogen). On the other hand, temperature higher than 55°C is fatal to all the organisms. Exposure to a temperature of 55°C for half an hour will kill all the pathogenic organisms, except the spores, if there be any. Spores, in their turn, are very resistant to heat. They may even withstand boiling for some time. There are some organisms present in the dung with the optimum temperature of 60°-70°C. These are known as *thermophilic bacteria.*

2. Oxygen.

Most pathogenic organisms require oxygen for their growth. There is, however, a class of organisms which will live only in the absence of free oxygen and give out carbon dioxide during their activity as a result of true respiratory exchange. These organisms are called *anærobic organisms.* Those which require oxygen for their growth are known as the *ærobes.* There are groups intermediate between them which are termed *facultative organisms.*

Facultative anærobes are organisms which are *ærobes* (*i.e.*, which live best in the presence of oxygen), but can also live in the absence of oxygen. Most cocci and intestinal organisms belong to this group.

Facultative ærobes are organisms, which, strictly speaking, are anærobes (live best without free oxygen), but can grow in the presence of oxygen.

True anærobes are the B. tetani, B. œdematic maligni, etc. These organisms generally live in dust and soil in the form of spores. When these spores are introduced in the tissue, they will multiply according to the amount of oxygen available, and will produce toxins. Thus, if the spores or the organisms enter into the blood, there will often be no growth, as the blood is very rich in oxygen. In the tissues, again, on account of large amount of oxygen present, they will not grow, unless the tissues are devitalised or necrosed. Hence, the infection of septic wounds with these anærobic organisms is so common. They produce very powerful toxins which are absorbed and give rise to various forms of diseases.

3. Light.

Light, either direct or diffuse, has the property of killing bacteria. Sunlight is thus a powerful bactericide. Bacterial cultures exposed to sunlight will be destroyed, so that it is essential to see that no light can enter the incubator during the growth of bacterial cultures. The bactericidal property lies mostly with the violet or the chemical rays of the spectrum. Infectious materials and discharges from patients suffering from cholera, plague, typhoid, etc., can be very easily disinfected by exposure to direct sunlight.

Any other powerful light, such as electric light, ultra-violet rays, exert powerful action in destroying bacteria. These are largely used in bacterial affections of the skin especially in lupus.

INFLUENCE OF ELECTRICITY.—There is no direct action on bacteria. Protozoa are killed by strong shocks. When a current is passed through a neutral liquid medium, most protozoa and vaccinia virus collect about the cathode, and most bacteria assemble behind the anode.

Röntgen rays have little effect, but radium and radio-active substances show a slight inhibiting effect on bacterial growth.

4. Moisture.

All bacteria are killed if dessicated. Moisture is essential for the growth of the organisms. Spores resist the drying process for a considerable extent. Anthrax spores will remain alive for several years, if kept dry, but, on the other hand, they will be able to resist longer, if kept moist.

5. Association with other Micro-organisms (symbiosis and antagonism).

Some organisms favour the growth of other organisms if allowed to grow together; whereas, in other instances, one destroys the other, if both are present in the culture or in the tissues. It has been observed that if B. tetani is inoculated into an animal in pure culture, tetanus does not take place. This is due to the fact that the tissue, being rich in oxygen, does not allow the tetanus bacillus to grow and produce toxin, as the organisms are anærobic. It staphylococcus or any other ærobic organism is introduced simultaneously with the B. tetani, the coccus absorb the available oxygen from the tissue, rendering it suitable for the growth of the B. tetani (*symbiosis*). On the other hand, B. pyocyaneus has got a very destructive influence on the growth of B. anthracis, if allowed to grow together (*antagonism*).

6. Nutrient Media.

Most of the pathogenic organisms can be grown on artificial culture media. Some amount of protein and salt must be present in the culture medium for the proper growth of the bacteria. Meat extract, peptone, etc., are the proteins commonly used for the media. The reaction of the media plays a very important part in the successful growth of bacteria. Most bacteria grow in slightly alkaline or neutral medium. Traces of acid in the medium may be quite sufficient to inhibit the growth, or even kill the organisms. Marked alkalinity is also unfavourable to most of the bacteria excepting only the cholera vibrio. Dieudonne's medium, which is composed of blood-alkali-agar, will allow only the cholera vibrio to grow, the alkalinity of the medium preventing the growth of all other organisms.

Of the salts, sodium chloride is commonly used. Potassium, calcium or magnesium salts may also be used.

Some organisms prefer some particular chemicals for their proper growth. For example, tubercle bacilli will grow in a medium containing glycerine; B. pestis, although it grows in other media, produces a characteristic stalactitic growth in ghee (clarified butter) or fat broth. B. influenza requires a medium containing blood, and, meningococcus, some tissue fluid such as the ascitic fluid for their growth.

THE COMMON NUTRIENT MEDIA

Liquid Media

1. Broth, or Peptone Broth, or Bouillon.

This is a *liquid medium*, and, is the basis of all other culture media. It is composed of as follows:—

Meat extract—1,000 c.c.
Sodium chloride—5 grms.
Witte's peptone—10 grms.

Mix and boil gently, until, all the substances are dissolved completely. The mixture is slightly acid, which is injurious to the growth of bacteria. Neutralise with saturated solution of sodium carbonate and add a few drops more until the reaction is slightly alkaline (pH 7.3 to 7.6). White of an egg may be used to clarify. Filter until the fluid is clear and light-yellow in colour. Sterilise in the steam steriliser. If still turbid, filter again, and sterilise. The broth must be perfectly clear.

The *meat extract is prepared as follows*:—

Cut up 500 grams of lean beef into small pieces, add 1,000 c.c. of distilled water, mix thoroughly, and keep in a cool place for 12-24 hours. Beef heart, if fresh, is more satisfactory. In hot weather keep in ice chest. Strain the fluid into a flask, and sequeeze until a litre of meat infusion is obtained.

This is used in the above formula. Instead of preparing meat extract, one can get the same from the market as Lemco (Leibig's extract of meat). 2 grams of lemco is mixed with one litre of water. The reaction of the medium prepared from lemco is less acid than when meat infusion is used. Meat infusion medium is almost colourless, whereas lemco medium is of brownish colour.

Other liquid media are the following:—

2. Glucose Broth.

This is prepared by adding 1 or 2 per cent. of glucose to the above broth.

3. Glycerine Broth.

This is prepared by adding 6 per cent. of glycerine to broth.

4. Peptone Water (Durham's).

This consists of 2% peptone with 0.5% sodium chloride dissolved in distilled water. The reaction of the medium is alkaline, and hence specially suitable for the growth of cholera vibrio. In this medium, cholera vibrio grows very rapidly, and, more vigourously than other fæcal organisms. It grows on the superficial layers, with the formation of a pellicle. The other fæcal organisms grow slowly and in the deeper layers.

This medium is also used to indicate the production of *indol.* This is done by adding a few drops of concentrated sulphuric acid in the media after allowing the organisms to grow for 24-48 hours.

5. Fermentation of Sugars by Organisms.

This can also be tested by adding litmus and sugars to the medium. " Sugars " include a variety of carbohydrates which are fermented by different organisms with production of acid with or without gas. The commonly used sugars are glucose, lactose maltose, mannite, saccharose, inulin, etc.

6. Trypsin Broth.

This is prepared by adding 1% of liq. trypsinæ co. to the broth. Incubate for three hours at 37.5°C.

Test the extent of trypsinising by adding to 5 c.c. of the broth 1 c.c. of 5% copper sulphate, mix thoroughly, and add 5 c.c. of normal caustic soda solution. A pink colour indicates that the process has continued fairly, and a bluish purple colour will indicate that more trypsinising is required. Add glacial acetic acid to render it slightly acid. Boil for some time, and allow to cool overnight. Take off the clear fluid, alkalinise and autoclave.

Solid Media.

1. Agar-Agar. (Also known as ordinary agar or nutrient agar).

This is the commonest culture medium used in the bacteriological laboratory. Agar is a form of carbohydrate derived from stems of seaweed, and is used in the powder form. This has the property of being soluble in boiling water, and solidifying on cooling. This solid medium melts again when the temperature is raised to nearly 100°C., and will not solidify again until the temperature is lowered to about 40°C. This property is advantageously utilised in the preparation of blood-agar or serum-agar media, as the agar-agar may be cooled much below the coagulation point of serum (70.5°C), and still remaining fluid. It is perfectly solid at 37.5°C, and hence most suitable for all purposes.

It is composed of 2% agar with bouillon (agar 2 grams, bouillon 100 c.c.). Boil bouillon, gradually add the agar until the whole is dissolved, make the reaction slightly alkaline with caustic soda solution, filter and sterilise in autoclave.

2. Glycerine-Agar.

This consists of 6% glycerine dissolved in melted agar-agar. This medium is specially suitable for B. tuberculosis.

3. Blood-Agar.

This consists of one c.c. of blood added to melted agar-agar at 55°C and allowed to solidify by cooling.*

4. Glucose-Agar.

This consists of 2% glucose with agar-agar. Litmus may be added with advantage.

5. Trypagar.

This consists of 2% agar in trypsinised broth and 0.215 grm. of calcium chloride per litre, neutralised, and autoclaved.

6. Ascitic or Hydrocele Fluid Agar.

Add 3 to 5 c.c. sterile ascitic or hydrocele fluid to melted agar at 40°-42°C. Slant the tubes and allow to solidify. Incubate at 37.5°C to determine whether the medium is sterile.

7. Salt-Agar.

Add 2 to 5 per cent. NaCl to ordinary agar. This is specially suitable for involution forms of B. pestis.

8. Gelatin Medium.

Gelatin melts at about 24°C., and so is absolutely useless as a solid medium in the tropics. Further, most of the organisms will grow best at 37.5°C (blood heat) which is much above the melting point of gelatin.

Liquefaction of gelatin may be determined by the following method.—Allow the organisms to grow in gelatin tubes for three days at 37.5°C. Put the tubes in ice or refrigerator. No solidification takes place if it has already been liquefied by the organisms.

Special Culture Media.

Media Containing Blood.

Specially suitable for hæmophilic organisms. Serum and blood media are divided into two classes:—

- (*a*) Where the blood or serum is coagulated by heat, resulting in the formation of a solid medium.
- (*b*) Where the blood or serum is added to an ordinary medium in liquid form.

1. Blood-Agar.

See above.

2. Inspissated Blood Serum.

The medium is used for cultivation of tubercle bacillus. Blood is drawn and kept for the clear serum to separate. The serum is sterilised by the process of fractional sterilisation at low temperature and inspissated.

3. Loeffler's Blood Serum.

Commonly used for the cultivation of B. diphtheria and other organisms. The medium is prepared by adding three parts of sheep's serum to one part of glucose broth and sterilised for three days at a temperature of 60°C.

4. Dieudonne's Blood-Alkali-Agar Medium.

This is used to isolate cholera vibrio. It has the property of inhibiting the growth of all the intestinal organisms except cholera vibrio.

This is prepared by adding equal parts of defibrinated ox blood and normal caustic soda solution. This is sterilised in a Koch's steriliser. Before use, three parts of this solution is mixed with seven parts of melted neutral agar-agar.

Maitra and Basu's Taurocholate-Agar Medium.

This is most suitable for isolation of cholera vibrio. This is prepared by adding $\frac{1}{2}$ per cent. of sodium taurocholate to ordinary agar of pH 7.4, from which plates are made.

Medium Containing Egg (Dorset's egg medium).

This is generally used for the cultivation of B. diphtheria and B. tuberculosis. Beat up the contents of four eggs and add 25 c.c. of water, mix thoroughly, filter, and autoclave. The medium can, with advantage, be rendered pink, by adding a little basic fuchsin.

Medium Containing Milk.

Make the reaction of milk neutral, steam for 15 minutes, allow to stand for 24 hours; siphon off the middle portion of the milk, leaving the cream and sediment behind, add litmus, and put in tubes containing Durham's tube. Sterilise by steaming on three successive days for 15 minutes.

Potato Medium.

Peel a potato. Cut a cylindrical portion with a cork borer, divide into two pieces diagonally; put each portion in a test tube, and sterilise in autoclave.

Cooked Meat Medium.

Take a portion of meat free from fat, and add equal weight of water (as many c.c. of water as there are grammes of meat). Heat to 40°C for half an hour, then boil, and autoclave for 20 minutes. It is advantageous to put liquid paraffin or vaseline in the tube before sterilisation, as it floats on the top and keeps the medium anærobic.

Media Used to Differentiate the Members of Coli-typhoid Group.

The constituents of these media are the following:—

(*a*) Chemicals which have the property to inhibit the growth of all other bacteria except the typhoid-coli group.

These are bile salts, crystal-violet, brilliant-green, picric acid, etc.

(*b*) Sugars which bring out the special characters of the suspected organisms. Lactose is commonly used.

(*c*) An indicator—either litmus, neutral red or acid fuchsin.

(1) MacConkey's bile salt medium.

This consists of bile salt, lactose, neutral red and agar in the following proportion:—

Sodi. taurocholate—5 grams; Peptone—1.5 grams; Lactose—3.5 grams; Agar—1.5; Neutral red (1 per cent. sol.)—about 0.50 c.c.; Tap water—100 c.c.

Dissolve peptone and the taurocholate in water, filter, add agar and egg white, autoclave, filter through lint, add neutral red and lactose. Add NaOH until colour becomes reddish-brown. Sterilise in Koch for 3 successive days.

(2) Conradi-Drigalski Medium.

This consists of crystal violet, lactose, litmus and agar.

(3) Endo-medium.

(4) Fawcus's Picric Acid and Brilliant-green Medium.

Noguchi's Medium for Treponema

Take 10 c.c. of 2% slightly alkaline agar in a test tube, melt and then cool up to 50°C., add 5 c.c. ascitic or hydrocele fluid, and put a small piece of fresh sterile rabbit's kidney at the bottom of the tube. After solidification of the medium, put about 5 c.c. of melted paraffin, in order to seal the tube from any access of oxygen. The fresh animal tissue also removes oxygen from the deeper layers of the culture medium, thus ensuring anærobiasis, which is essential. Inoculate the bottom of the tube by means of a capillary pipette.

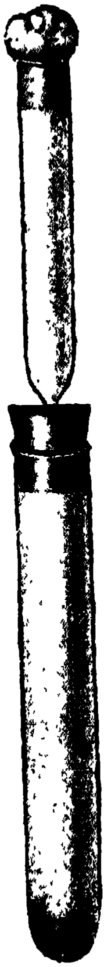


Fig. 30.

Noguchi's tube for cultivation of Spirochaetes.

Culture of Anærobic Organisms

Principle.—All the ordinary culture media absorb, some amount at least, of free oxygen, either during the preparation or after; hence, for the cultivation of the anærobic organisms, it is essential that the medium is free from any free oxygen. The following are the methods to make the media oxygen-free:—

1. Expulsion of the absorbed air by heating, or by passing an inert gas, *e.g.*, hydrogen, through the media.
2. Keeping the media in an atmosphere of hydrogen, nitrogen or some other inert gas.
3. Absorbing the oxygen from the air surrounding the media by (*a*) vacuum, (*b*) pyrogallic acid and caustic potash, (*c*) reducing body, *e.g.*, glucose.
4. Placing small piece of fresh sterile animal tissue in the liquid culture medium. (Tarozzi's Method.)

Liborius's Method.

A test tube is filled to about half its height with glucose agar-agar, and the contents boiled for a few minutes in order to expel oxygen from the medium, and then placed in water bath at 38°C. for a few minutes. It is inoculated with the material, and then rapidly solidified in cold water. The anærobic organisms grow in the deeper layers. Instead of keeping in water bath, the media may be allowed to solidify, and then inoculated deep with a pipette or a platinum wire, the so-called ***deep stab culture***. The track of the pipette may be sealed by heating the upper part of the tube, or adding some melted medium.

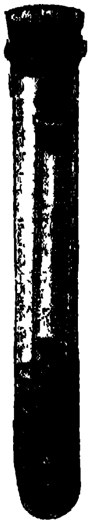


Fig. 31. Buchner's tube.

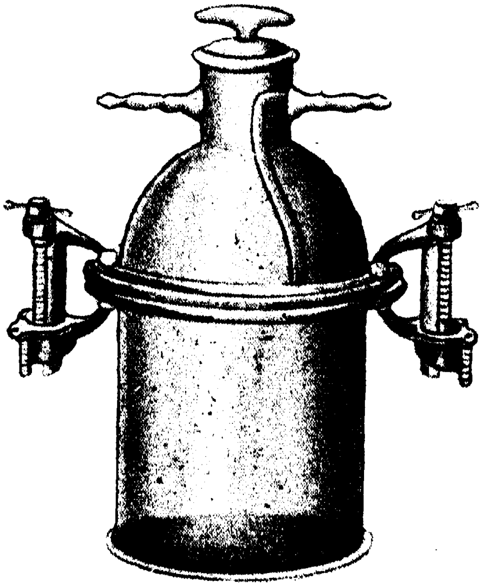


Fig. 32. Novy jar.

Buchner's Method.

The inoculated culture tube is placed in a bigger test tube. A quantity of pyrogallic acid is put into the bigger tube, the required quantity of the alkali is run in and the opening rapidly closed air-tight.

Novy Jar.

The culture media are put into the jar in a beaker. The air can be withdrawn by means of an air pump or by alkali-pyrogallic acid mixture. The joints of the apparatus must be well smeared with vaseline.

Bulloch's Apparatus.

This consists of one bell-jar which is inverted over a shallow glass dish of slightly lesser diameter. The bell-jar is furnished with two stoppered appertures at the top, one of which is in communication with the glass dish by a long tube. About four grms. of dry pyrogallic acid is placed on one side of the glass dish, and the culture media in a beaker on the other side. The bell-jar is inverted over the dish in such a way that the long tube from the top is placed over it and the rim secured air-tight by vaseline. Fit a rubber tube to the stopper which has the long glass tube communicating with the dish below, and close the stopper. Connect up the other stopper with short tube with an air pump, make partial vacuum in the jar with the pump, and then close the stopper. Dip the end of the rubber tube into a vessel containing a solution of 109 grms. solid caustic potash dissolved in 145 c.c. of water, and open the stopper connected with it. The fluid is sucked into the shallow dish, and, coming in contact with pyrogallic acid, produces potassium pyrogallate, which absorbs the free oxygen from the air. The whole apparatus is placed in the incubator. It is advantageous to pass a current of hydrogen from a Kipp's apparatus through the jar, before aspirating with the pump.

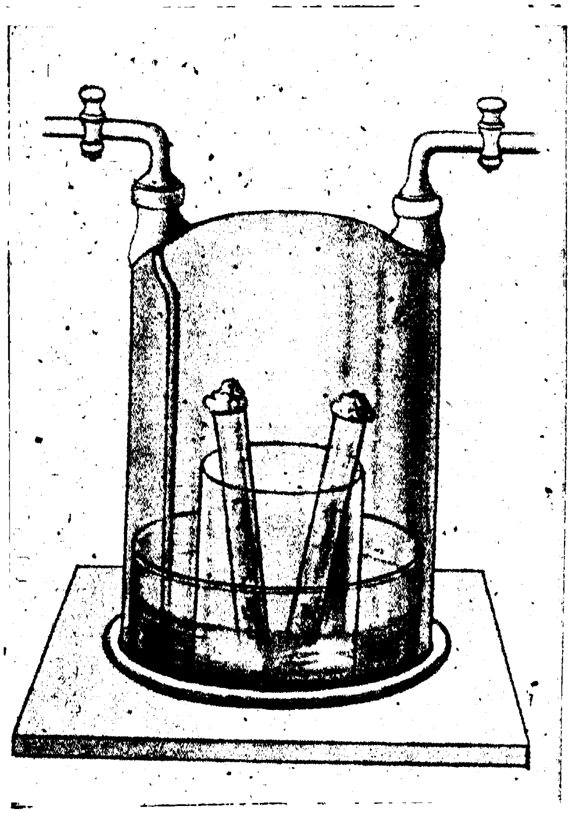


Fig. 33. Bulloch's apparatus.

CHAPTER VIII

STUDY OF THE BACTERIA

The complete study of bacteria comprises the study of the following characters:—

(*a*) **Morphology;**
(*b*) **Biology;**
(*c*) **Staining;**
(*d*) **Cultivation;**
(*e*) **Serological Reaction;**
(*f*) **Animal Experiments;**
(*g*) **Pathogenesis.**

(A) Morphology

The Structure of Bacteria.

The structure of bacteria consists of a cell possessing more or less homogeneous protoplasm, and a cell wall.

The Protoplasm is more or less uniform throughout, except, in some instances, where granules or spores are present. The granules are often metachromatic, and have affinity for special stains, *e.g.*, in B. diphtheria.

The *cell wall* is distinct, and is composed of cellulose.

Capsule.—In some instances, as in pneumococcus, the organism is surrounded by a capsule which is best observed in the tissues.

1. SIZE.—Bacteria are all very minute organisms. The size of bacteria is studied either in the unstained or in the stained preparation. The size is measured by the micrometre scale, or by comparison with the size of a red blood corpuscle. The unit of measurement is a *micron* (wrongly termed micromillimetre) which is 1-1000th part of a millimetre and is designated by the Greek letter μ. A rough idea of the size of the bacteria can be gained by comparing it with that of a human red blood cell which is about 7 μ in diameter. The largest bacillus recorded is B. butschlii (50-60 μ long and 4-5 μ wide), while some are so minute that they are invisible even with the highest power of any microscope—the so-called ultramicroscopic or filtrable virus. Their existence is known by the fact that their

filtrates, after passing through the Pasteur-Chamberland filter, are infectious.

2. Shape and Arrangement.

Bacteria differ in shape from one another, being dots, rods, etc.

All dot-shaped organisms are the *cocci.*

All rod-shaped organisms are the *bacilli.*

All cork-screw shaped organisms are the *spirilli.*

Migula uses the term *vibrio* to indicate curved rods with one or two polar flagella, and *spirilla,* to indicate those with a large number of polar flagellæ.

Arrangement mainly depends on the method of division. If the organism divides in one axis only, and the individual segments are kept attached to each other by means of their envelope, it forms a chain as in *streptococcus.* When they are grouped in pairs, they are known as *diplococci.* When the division takes place in two planes at right angles to each other, the organism is known as a *tetracoccus.* If the division takes place in three planes, it is known as *sarcinæ.* If they divide in any axis forming masses or bunches, they are called *staphylococci.*

3. Motility.—This is best seen in an unstained liquid film (hanging drop preparation). A young fluid culture is preferable. Take a platinum loopful of the fluid culture on a slide. Gently put a cover-glass on it. It is an advantage to include a few small air bubbles under the cover-glass, as they help, specially the beginner, a good deal, during the focussing of the film. Focus one small air bubble, or the margin of a large one. Turn the high power lens to focus the margin of a bubble where the bacteria can be easily observed. The margin of the cover-glass may be covered with vaseline so as to avoid evaporation and production of currents in the liquid.

The true motility of an organism should be differentiated from—

(*a*) *Brownian Movement.*—This is a vibratory to-and-fro movement without any changing of position. This is found in the case of any fine floating particle.

(*b*) *Movement due to Currents in the fluid.*—In this, all the bacteria are seen to move in the same direction. *Dark ground illumination* is the best to study the bacteria, specially the spirochætes, as they appear as brightly illuminated and highly refractile objects on a dark background. This requires a special silvered substage condenser, which allows only the peripheral rays to pass through the film, at the same time preventing the central rays to pass. A most critical illumination is absolutely necessary, preferably, a brilliant electric arc. This is allowed to pass through a condenser—a round flask filled with distilled water, coloured lightly with copper sulphate crystals, which shuts off the heat rays and gives monochromatic light. In case of oil-immersion lens (1-12th objective), a funnel stop is absolutely necessary, as it produces better definition by cutting off the peripheral rays. Slide and cover-glass must be perfectly clean, and the latter must be the thinnest available. A thin uniform layer of cedar wood oil, free from any air bubble, should be placed between the condenser and the under-surface of the slide.

The motility of the organism can be beautifully observed by this process.

4. FLAGELLA.—All motile bacteria possess fine thread-like prolongations of protoplasm known as *flagella*. They are of varying numbers and sizes. They require special staining process for their demonstration, as they are invisible, even in ordinarily stained specimens.

The following terms are used to denote bacteria with flagella—

Bacteria with no flagella	*atricha.*
Bacteria with one flagellum	*monotricha.*
Bacteria with two flagella	*amphitricha.*
Bacteria with many flagella	*peritricha.*

N.B.—Bacteria with flagella are to be differentiated from the flagellates. Flagellates are protozoa possessing flagella, *i.e.*, *animal parasites*, whereas the former are bacteria, and hence, *vegetable parasites*.

5. SPORES.—These are bodies developed in certain bacilli representing the resting stage of the organism. These are very resistant, and are produced only when the organism is under unfavourable condition as regards food supply or environment. These originate as highly refractile minute round dots in the protoplasm of the bacilli, which gradually enlarge and sometimes become broader than the original cell.

The spore consists of a thick membrane enclosing a mass of protoplasm. It is supposed that the covering membrane of the spore gives it the property of resisting external agencies, whereas, in other instances, the covering is absorbed, or disposed of otherwise, when placed in a suitable culture medium.

Usually one bacillus produces a single spore, which, again, produces a single bacillus, which eventually divides, with the production of a large number of bacteria.—Spores are incapable of direct multiplication, and are non-motile.

Spores may be (*a*) *central*—occurring in the middle of a bacillus as in B. anthracis;(*b*) *terminal*—occurring at the end of the bacillus as B. tetani; and (*c*) *subterminal*—occurring between the middle and the end of a bacillus as in B. œdematis maligni.

Spores require special staining process for their demonstration.

Involution forms.—It is occasionally seen in cases of staphylococcus and streptococcus, etc., that some of the organisms are bigger and take deeper stain, but they are not resistant to heat and disinfectants like true spores. They represent degnerative and distorted forms of the organism. Transference to a suitable culture medium often restores their normal size and shape. Old cultures of staphylococci often show distorted bacillary forms, and old cholera culture shows a large number of sickle-shaped bodies or granular material. The involution forms of B. pestis are quite characteristic, specially when cultivated in agar with high concentration of salt. Variation in size and shape of bacteria is called *pleomorphism*.

6. CAPSULE.—Some bacteria possess a capsule surrounding the organism.

7. BRANCHINGS.—Some organisms, *e.g.*, B. tuberculosis, diphtheria, often show branchings. These are often regarded as the connecting link between bacteria and higher fungi, *e.g.*, streptothrix.

8. MULTIPLICATION.—Multiplication takes place by transverse fission.

Cocci divide in one or more planes, as follows:—

(*a*) in one plane—division regular—(*i*) in the form of chain, *streptococcus*, (*ii*) in pairs, *diplococci*.

(*b*) in two planes—(*i*) division irregular, appearing like bunches of grapes, *staphylococci*, or (*ii*) division at right angles in two planes, *tetracocci*;

(*c*) in three planes—like bails of jute—*sarcinæ*.

Bacilli divide in one plane only.

Weight of Bacteria.—Rubner has found that the specific gravity of bacteria varies from 1.038 to 1.067. According to Kendall, the weight of one B. coli is about 0.000000000613 milligram. The weight of one red blood cell is about 0.00008 milligram. Thus, one red blood cell is 50,000 times heavier than one B. coli.

Chemical Composition of Bacteria.—The bacteria are composed mainly of water and protein material. Ruppel gives the following percentage composition of B. tuberculosis:—

Water 85.00; Proteins 8.50; Extractives 4.00; Ash 1.400; Residue 1.10. He also analysed 100 grms. of dried B. tuberculosis with the following results:—

Nucleic acid 8.50 grams. Nucleoprotamin 24.50 grams. Nucleoprotein 23.00 grams. Albuminoids, keratin, etc. 8.30 grams. Fats and wax 26.50 grams. Ash 9.20 grams.

(B) Biology

Temperature Reactions.

All organisms, with a few exceptions, grow best at the human body temperature (37.5°C.) which is known as the *optimum temperature*, and growth usually ceases below 10°C. and above 42°C. They are not killed by freezing, but a temperature above 55°C. for half-an-hour is fatal to all organisms, excepting the spores. Spores can even resist boiling.

Relation with Oxygen.

Free oxygen is essential to the growth of many organisms, whereas there are others which will grow in presence of free oxygen.

Organisms which require free oxygen for growth are called *ærobes*.

Organisms which cannot grow in the presence of free oxygen are known as *anærobes*.

Organisms which are anærobes but can grow in the presence of oxygen, are known as *facultative ærobes*.

Organisms which are ærobes but can grow in the absence of oxygen, are known as *facultative anærobes*.

(C) Staining Reactions

Preparation of a Smear.—Spread a very small amount of the material to be examined on a slide by means of the platinum loop in the form of a very thin film, allow it to dry, and fix it by passing over the flame two or three times.

(*i*) The following basic aniline dyes are commonly used:—

Methylene blue, fuchsin, gentian violet, safranin, bismarck brown (vesuvin), methyl violet, etc., in which the organisms will be stained blue, red or violet respectively. These dyes are derived from the coal tar product aniline, and hence they are termed *aniline dyes.*

According to the presence of the basic or acid molecule, the dye is called *basic aniline dye* or *acid aniline dye.* The basic dyes have affinity for nuclear structures, whereas the acid dyes stain the cytoplasm.

Acid aniline dyes are not suitable for bacteria. They are generally used in bacteriology for the purpose of counterstaining. They are eosin, acid fuchsin, congo red, etc.

To prepare these stains, make a saturated alcoholic solution of the stain which will keep indefinitely. For use, take one part of the saturated alcoholic solution, mix with ten parts of 5 per cent solution of carbolic acid and shake well.

(*ii*) *Neisser's Stain*:—

Methylene blue, 1 grm.; Alcohol, 12 c.c.; Acetic acid, 5 c.c. Water, 10 c.c.

(*iii*) *Flagella Staining*:—

Rosanilin hydrochlor, 1.5 grm.; Aluminium chloride (cryst), 18 grm.; Zinc chloride, 10 grm.; Tannic acid, 10 grm.; Alcohol (60%), 40 c.c.

Dilute 1 in 5 just before use and filter on the slide.

Make the slide fat free by thoroughly washing with soap and water and then passing several times over the flame. Take the smear on the slide. Stain with the above stain for one minute. Treat with cold carbol-fuchsin for 5 minutes. Wash and dry.

(*iv*) *Spore Staining*.—*See* under B. anthracis.

(*v*) *Gram's Staining*—GRAM-POSITIVE organisms will be stained deep violet, *i.e.*, they take the basic dye, whereas the GRAM-NEGATIVE organisms will be stained red.

This is due to a fundamental difference in their chemical structure. The strongly Gram positive bacteria possess a chemical structure which consists of substances giving it a markedly acid reaction, *i.e.*, the H ion concentration is above the iso-electric point. The Gram negative organisms, on the other hand, possess substances having a markedly basic reaction, *i.e.*, the H ion concentration is below the iso-electric point. The Gram variable bacteria do not give a sharp reaction one way or the other because they possess iso-electric point very near the reaction of the staining solutions.

(*vi*) *Special Stains*:—
  (*a*) For acid fast organism and spores—Ziehl-Neelsen's method.
  (*b*) For diphtheria—Neisser's stain.
  (*c*) For capsule staining.
  (*d*) For flagella staining.

(D) Cultural Reactions

Collection of Material.

The vessel in which the material is to be collected must be absolutely free from any bacteria as well as from any antiseptic. It must be sterilised by boiling, or according to the methods given under sterilisation. Presence of any antiseptic will destroy the organism present, and there will be no growth.

The material may be collected by (*a*) a platinum loop, (*b*) a sterile swab, or, (*c*) aspirated with a sterile syringe.

Sterilisation

Bacteria are present everywhere—in the constituents of the culture media, in the test tubes or other vessels used as containers for the media, in the platinum wire, or other instruments used to collect the material for cultivation, as well as on the part of the body in the neighbourhood of the material to be examined and cultivated. It is essential that no extraneous organisms (contamination) should get any access to the culture media while the characters of any species of the organism are under study. This is effected by destroying all the bacteria present in things which come in contact with the culture media or the material for study. This process of destruction is known as *sterilisation.*

Methods of Sterilisation.

The nature and composition of the various things for sterilisation varies a good deal, and hence, one method cannot be used for all purposes. Thus, a platinum wire can be very easily sterilised in the naked flame of a gas burner, but that is imposible in the case of various other substances. Sterilisation can be effected by means of antiseptics, but they are useless in bacteriological work, as they interfere with the subsequent growth and isolation of the bacteria under study. The best means is heat, whether dry or moist, with or without increased pressure. In order of effectiveness, moist heat exerts greater effect than dry heat, and moist heat, under pressure, is more effective than that without pressure.

1. Sterilisation by Dry Heat

(*a*) *Holding Over Direct flame.*—This is most commonly used in case of sterilisation of platinum needle. The outer part of the cotton plug used for the culture tube is conveniently sterilised by burning it over the flame. Surgical instruments for autopsy of an experimental animal, *e.g.*, scalpel or parts of the forceps can also be sterilised by this method if contamination is suspected. Glass articles, *e.g.*, slides, cover-glasses may also be sterilised by this process, care being taken not to crack or melt the glass.

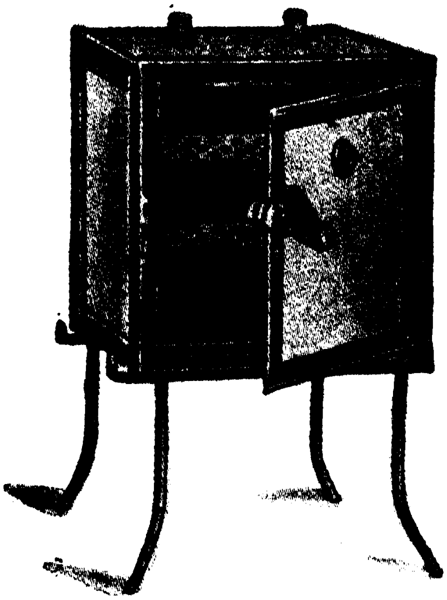


Fig. 34. Hot air steriliser.

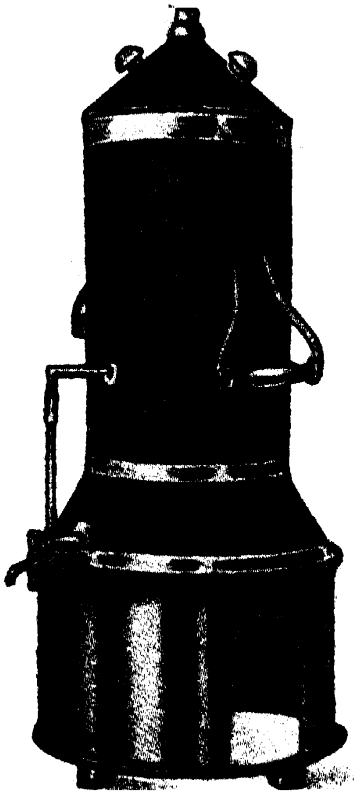


Fig. 35. Koch's steam steriliser.

(*b*) *Sterilisation by Hot-Air Steriliser.*—The hot-air steriliser consists of a double-jacketed chamber of strong sheet-iron. The bottom is generally made of copper. Two or more perforated moveable shelves are provided inside the chamber, for putting in the articles for sterilisation. A bunsen burner is placed at the bottom, and the hot air passes between the two jackets from below upwards. On the top, there is a perforated slide, through which the hot air escapes, and which serves the purpose of regulating the current of air. There are two other holes at the top of the apparatus, one for the thermometer, and the other for a thermo-regulator, if necessary. This steriliser is generally used in the case of articles *e.g.,* test tubes, pipettes glass flasks, petri dishes, cotton wadding, etc. Flasks, tubes and other open vessels are closed with cotton wool plug, and the petri dishes, pipettes, etc., are wrapped with old newspaper before being put inside the steriliser. The sterilisation of culture media is not suitable by this process, as they get dry by the heat. Sudden heating or cooling must be avoided, as they are liable to break the glass. For this reason, the apparatus must be allowed to cool before it is opened.

A temperature of at least 150°C. for not less than one hour is necessary for proper sterilisation by this method.

2. Sterilisation by Moist Heat

(*a*) *By Boiling.*—This is generally used in the case of sterilisation of instruments before and after autopsy, or to sterilise water for any purpose. For instruments, it is advantageous to put some soda carbonate in the water. This removes grease from the instruments and also raises the boiling point. Boiling for one-and-a-half hour is generally sufficient to kill spores, but some are so resistant that they withstand continued boiling for 6 hours. The inside of a test-tube may be sterilised by boiling water in it.

(*b*) *By Steam under Ordinary Atmospheric pressure.*—This is generally done by "Koch's Steam Steriliser" or "Arnold Steam Steriliser." Arnold's apparatus is smaller, and gets more rapidly heated than that of Koch.

Koch's apparatus consists of an all-metal cylinder, the outer surface of which is covered with asbestos which is a bad conductor of heat. It is covered by a lid with a hole for the thermometer. A tap is fitted at the botton for the withdrawal of water. Inside, there is a perforated chamber which lies a little above the surface of water which is three or four inches deep. Heat is applied at the bottom. The steam will surcharge the culture media put on the diaphragm, the temperature of which will be raised to 100°C. as it is surrounded by steam and no evaporation can take place from the media. Steaming for one and a half hour is quite sufficient for sterilisation. For organisms with spores, fractional sterilisation with this apparatus is the best.

(*c*) **By Steam under High Pressure.**—This is done by means of **Autoclave** and is the best and most rapid method of sterilisation.

The *autoclave* consists of a strong gun-metal cylinder with a top of the same metal which is fixed to the cylinder by screws and nuts so as to withstand high pressure. The lid is provided with a pressure gauze (often indicating both pressure and temperature), a safety-valve and blow-off pipes. A thermometer may or may not be present. The safety-valve is so adjusted that the desired pressure is maintained, and when it exceeds the mark, it blows off spontaneously, thus avoiding the excess of pressure. The blow-off pipe is used to drive off the air contained in the apparatus before it becomes filled up with steam. It is also used to relieve pressure from inside by allowing the steam to escape through it.

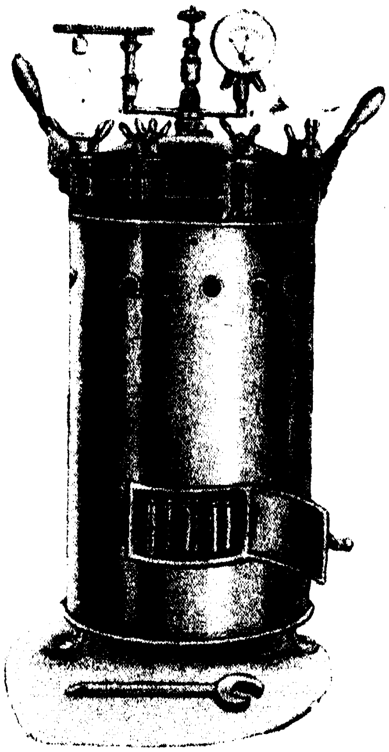


Fig. 36. The Autoclave.

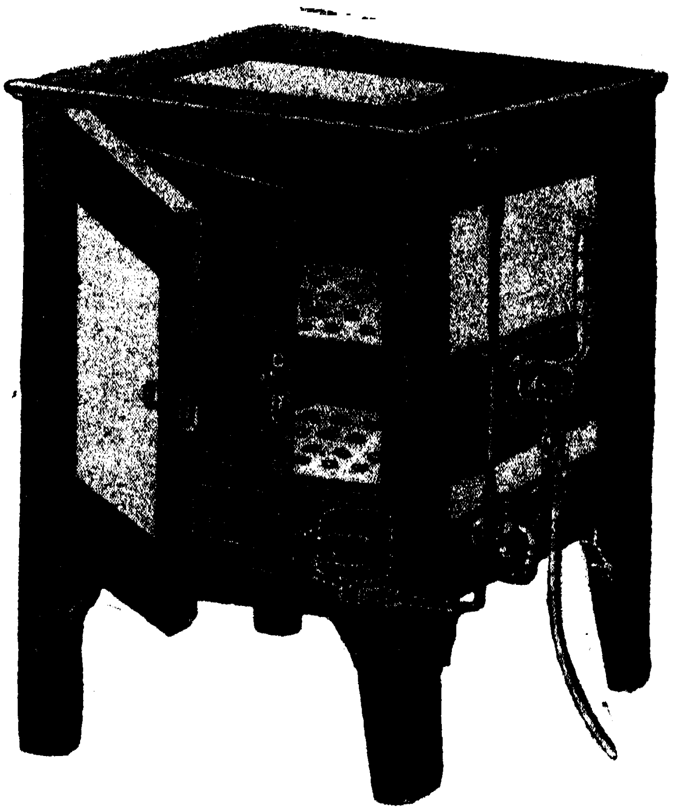


Fig. 37. The incubator.

The contents of the apparatus are more or less the same as in Koch's steriliser, *i.e.*, a collection of water of a depth of about one inch at the bottom with a perforated diaphragm supported above the level of water. The source of heat is generally from several Bunsen burners.

Use of the Apparatus.

See that the depth of water inside the apparatus is not less than an inch. Put the perforated diaphragm inside the cylinder, and place the materials for sterilisation on it. Put the cover on, and fix with the cylinder by screws and nuts. Apply heat at the bottom, unscrew the blow-off pipe, and wait until steam passes through it with considerable force, indicating that all the air present inside the apparatus has been replaced and expelled by steam. Closely tight the blow-off pipe. The pressure gauze will show increase of pressure. A pressure of about 30 lb is equivalent to the temperature of 120°C. Keep the apparatus at this temperature and pressure for 15 minutes and then put off the burners. Open the blow-off pipe when the pressure has been reduced to 0 so as to let off the excess of steam. Open the lid and take out the materials.

Precautions.

(*i*) See that all air from inside the apparatus has been expelled, otherwise, the pressure gauze will indicate the pressure of a mixture of air and steam, which would be misleading.

(*ii*) See that the water inside is not too small to be totally converted into steam, in which case it is liable to be superheated, so that the pressure gauze will give inaccurate indications.

(*iii*) Allow the apparatus to cool below 100°C.; otherwise, the relief of pressure through the blow-off pipe on opening the lid will cause the media to be blown off the containers.

(*d*) **By Fractional Sterilisation.**—It is known that exposure to a temperature of 55°C. for half an hour is quite sufficient to kill all bacteria excepting the spores. The spore-bearing organisms also will be killed, leaving the spores alive.

Advantage is taken of this fact in the sterilisation of spore-bearing organisms by exposing them to steam as in Koch's steriliser or by direct boiling for half an hour. This kills all the bacteria except the spores. This is kept on for 24 hours, by which time, many spores, being present in a favourable medium, develop into bacteria. This is again subjected to heating at 100°C. for half an hour on the second day. The same is repeated on the third day. It is found that sterilisation for three consecutive days, in the aforesaid manner, will kill all the organisms with the spores, as, during that period, all the spores have developed into bacteria and been destroyed, This process is known as *Fractional Sterilisation or Tyndall's Intermittent Sterilisation.* This method is specially used in cases of sterilisation of blood serum and gelatin. Blood serum will coagulate at a temperature above 55°C. Hence, it is impossible to sterilise it either in Koch's steriliser or in Autoclave, in the usual way. This is generally done by the process of fractional sterilisation at 55°C. for at least three consecutive days, preferably more. Gelatin, on the other hand, loses its property of solidification if exposed to the heat of an Autoclave or prolonged boiling or steaming; here, steaming for half an hour for three days will sterilise the medium.

Pasteurisation of milk is also done by the same process.

(e) Sterilisation at Low Temperatures.—This is generally used for killing bacteria for the preparation of vaccines, and is done in a water bath at a temperature of 60°C. for one hour. A higher temperature may diminish the immunising property of the vaccine.

(f) Sterilisation by Filtration.—Ordinary bacteria are too big to pass through the pores of Chamberland or Berkefeld filters, and so they can be filtered and a bacteria-free filtrate can be obtained. The pores of ordinary filter paper allow the bacteria to pass readily through them. If the bacteria be suspended in an albuminous fluid, the finely-meshed paper often filters the larger bacteria. Pasteur-Chamberland candles of unglazed porcelain or Berkefeld filters are used to filter the bacteria, but they are not absolutely impermeable to the smaller bacteria, especially certain spirochætes (leptospira icteroides). It is the width and not the length of the organism which determines their ability to pass through the filters. The maximum width for filtration is about 0.2 μ.

Incubation of the Culture.

This is generally done at blood heat, *e.g.*, a temperature of 37·5°C. The temperature must be continuous and uniform. This is generally done in an *incubator.* The incubator consists of a double-jacket chamber with water between the walls. The chamber is provided with a double door, the inner one being made of glass. The inside consists of two or more chambers separated by partitions. Gas is admitted through a regulator where there is a mechanism by means of which the gas is automatically decreased as soon as the desired temperature is reached and kept continuous at the same temperature.

(E) Serological Reactions.

When an organism, specially one of the typho-coli group, is introduced into an animal, either experimentally or in disease, the serum of the animal acquires the property of agglutination or clumping the culture of the same organism. This agglutinating property is specific, in this sense that the serum will agglutinate no other organism than the one which has been introduced into the animal.

(F) Inoculation of Animals.

Common laboratory animals are guineapig, rabbit, pigeon and mouse. Inoculations are made either subcutaneously, intravenously, intraperitoneally or into the anterior chamber of the eye. Mouse is used for pneumococcus, rabbit for staphylococcus and guineapig for the rest.

(*a*) *Subcutaneously*—(1) By a hypodermic syringe, if the material is fluid: (2) introducing the material in a pocket under the skin made by a pair of sterile scissors.

(*b*) *Intravenously*—In the ear vein of the rabbit.

(*c*) *Intraperitoneally*—(1) By a hypodermic syringe, if the material is fluid; (2) by means of laparotomy, if the material is solid.

(*d*) *Into the anterior chamber of the eye* (Cocainise the eye)—(1) By a hypodermic syringe, if the material is fluid; (2) in case of solid material, make a puncture with the knife through the cornea just in front of the corneoscleral junction. Allow the vitreous humour to flow out; introduce the material with a pair of fine sterilised forceps.

The following conditions are to be observed in the inoculated animal:—

(*a*) Temperature; (*b*) Attitude; (*c*)Loss of appetite; (*d*) Loss of weight.

During animal dissection, do not forget to (*i*) dissect with sterilised instruments; (*ii*) note the condition of the seat of inoculation and internal organs; (*iii*) make cultures from the heart's blood, spleen, liver, kidney or any exudate present; (*iv*) examine the spleen, liver and kidney histologically; (*v*) disinfect the animal, the post-mortem board and the instruments after the autopsy.

CHAPTER IX

SPECIAL BACTERIOLOGY

Points to Mention in Describing the Bacteria

1. *Source*:—
    - (*i*) In the human body—during life and after death.
    - (*ii*) In animal body—during life and after death.
2. *Morphology*:—
    - (*i*) Shape.
    - (*ii*) Arrangement—grouping in pairs, chains, clumps, etc.
    - (*iii*) Size—occurrence of involution forms.
    - (*iv*) Presence of motility.
    - (*v*) Presence of flagella.
    - (*vi*) Presence of spores, if present, whether terminal, central or subterminal.
    - (*vii*) Presence of capsules.
3. *Biology*:—
    - (*i*) Temperature reaction.
    - (*ii*) Relation with oxygen—ærobe, anærobe or facultative.
    - (*iii*) Pigment formation.
4. *Staining*:—
    - (*i*) With aniline stain, *e.g.*, methylene blue, whether even beaded, bipolar or showing metachromatic granules.
    - (*ii*) With Gram's method.
    - (*iii*) With special stain, if any.

Cultural Reaction:—
- (*i*) In agar (shape, colour, opacity and consistency of the growth).
- (*ii*) In broth (turbidity, deposit, pellicle formation).
- (*iii*) In other media.

6. *Biological Products*:—
    - (*i*) Fermentation of sugar.
    - (*ii*) Coagulation of milk.
    - (*iii*) Liquefaction of gelatin.
    - (*iv*) Change in the reaction of media.
    - (*v*) Formation of indol.
    - (*vi*) Formation of nitrites.
7. *Agglutination Reactions with High Titre Serum.*
8. *Inoculation Reactions.*
9. *Pathogenicity to Human Body.*

COCCI

STAPHYLOCOCCUS PYOGENES AUREUS

(Ogston 1881)

Source.—It is commonly present in the oral and intestinal contents, skin and conjunctiva. It becomes pathogenic only when it comes in abnormal circumstances.

Morphology.—A minute dot-like organism, usually grouping in masses or clumps, but may be in pairs or singly about 0.9μ in diameter, non-motile, without any flagella or spore, and with no capsule. They divide irregularly in two planes, in contrast to the single plane division of streptococci. Frequently they are seen inside pus cells, showing all stages of disintegration.

Biology.—It grows at temperatures from 18°C to 40°C, optimum being 37.5°C. It is very resistant to heat, desiccation, and disinfectants.

Staining Reaction.—It stains readily with all aniline dyes and is Gram-positive; there is no other special stain. In pus, some of those taken up by leucocytes may appear Gram-negative and may be mistaken for gonococci.

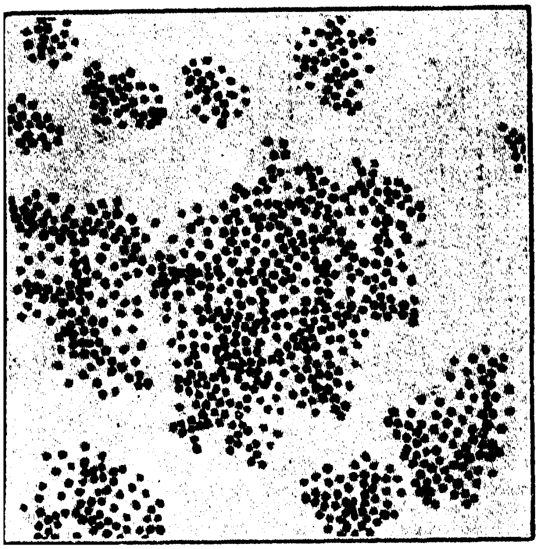


Fig. 38. Staphylococcus, smear from broth culture.

Cultural Reactions and Biological Products. AGAR-AGAR.—It forms a thick, opaque, moist, shining, cream-coloured growth, with sharply-defined margin. The colour of young culture is white, gradually becoming gold-yellow in 24 to 48 hours. The pigment belongs to the group of lipochromes. BROTH.—Turbid yellowish sediment at bottom. Old broth cultures contain a toxic substance which has got the property of disintegrating leucocytes and is known as *leucocidin.* BLOOD SERUM.—Same as agar-agar.. GELATIN STAB.—Growth takes place along the line of stab. Gelatin is liquefied, assuming a funnel-like form, the liquefied portion being turbid. POTATO.—Same as agar. MILK.—Coagulated with production of acid.

It produces *indol* but no *nitrites.*

The colonies of staphylococcus may be white, lemon yellow, orange or golden yellow. The most satisfactory method of examination of colour is suggested by Winslow who spread the loopful of culture, on white paper and noted the colour after it had dried. The colour develops in the presence of oxygen and anærobic growth may induce permanent loss of chromogenic powers.

Inoculation.—A culture given intravenously into a rabbit will produce septicæmia and death in less than 24 hours; sometimes this is delayed to 3 days. Kidneys, muscles and other organs show multiple abscesses and focal necrosis. These necrotic areas are usually microscopic in case of rapid death of the animal; otherwise, they are distinctly visible to the naked eye. The organism can be isolated in pure culture from the above abscesses or heart's blood.

Toxic Products.—(*i*) Leucocidin, which is a soluble toxin produced by the staphylococci both *in vitro* as well as *in vivo,* which affects both the leucocytes and the tissue cells by destroying them, (*ii*) hæmolysin, and (*iii*) endotoxins.

Pathogenicity.—In all localised abscess formation, staphylococcus plays an important part. In man, the staphylococci are the infective agents in various diseases, some trifling, some severe.

Primary infection.—These are usually the primary and the only organisms in boils, curbuncles, deep whitlows and osteomyelitis.

Secondary infection.--As secondary invaders they occur in any skin lesion involving loss of continuity of epidermis, (acne, eczema) also in nasal catarrh, broncho-pneumonia, genito-urinary affections (urethritis, prostatitis, cervicitis, pyelitis), endocardits, parotitis etc.

The affections caused by Staphylococcus are the following:—

Superficial and deep abscesses, boils, carbuncles, whitlows, endocarditis, osteitis, periostitis, osteomyelitis, peritonitis, pericarditis, meningitis, puerperal septicæmia, pyæmia, etc.

Pathology of lesions in man.—The typical lesion is essentially a local one. Soon after the onset, there is an emigration of leucocytes around the infected area. The toxin (leucocidin) produced by the organisms destroys many of these leucocytes and also tissue cells so that in typical lesion, there is a core of necrosed tissue surrounded by thick pus containing large number of dead and degenerated leucocytes.

In acute osteo-myelitis, the primary lesion is usually insignificant and often escapes notice. Polymorphonuclear leucocytosis occurs in acute staphylococcal infection.

INFLAMMATORY AND SUPPURATIVE CONDITIONS.—

These are produced by a variety of organisms. Although there is no one organism specific for these conditions, yet it is usual to find the same organism producing, according to circumstances, a variety of affections. Thus, suppuration, although commonly caused by staphylococci, may be caused by many other organisms, *e.g.*, streptococcus, pneumococcus, meningococcus, gonococcus, M. tetragena, B. pyocyaneus, B. of typho-coli group, B. proteus, B. mallei, actinomyces, etc.

OTHER VARIETIES OF STAPHYLOCOCCUS.—

Staphylococcus pyogenes albus.—These are less virulent than staphylo. pyogenes aureus and much less pathogenic to the rabbit. They differ from the above by the colour reaction found on solid cultures. The colour is white instead of golden yellow. They are commonly found in the mouth and the nose.

Staphylococcus pyogenes citreus.—Least virulent of all. Colonies are bright yellow, but otherwise resemble staphylococcus pyogenes aureus.

Staphylococcus epidermidis albus.—Probably the same thing as staphylococcus pyogenes albus This is always found on the epidermis and occasionally remain at such a depth that ordinary disinfection of the surface of the skin may not affect these organisms.

Staphylococcus cereus, albus and flavus.—Otherwise similar to the staphylococcus pyogenes aureus in all respects, colours on solid media are white and yellow, respectively. They are less virulent and do not liquefy gelatin.

The old classification of staphylococcus into S. aureus, S. albus, and S. citreus according to the colour of the colony, and S. epidermidis albus, differentiated by its failure to ferment mannitol, is now found to be useless. The classification into S. pyogenes and S. epidermidis, by Hine's Serological Absorption Test, is the most satisfactory.

Immunity.—Although antibacterial serum is generally unsuccessful in staphylococcic infections, still inoculation of killed cultures (vaccines) produces active immunity. A dose of 100-2000 million killed organisms subcutaneously may be given without any risk of general reaction. Besredka has found that a high degree of local immunity can be produced by intradermal injection of filtrates of staphylococcus cultures which he terms *antivirus*.

STREPTOCOCCUS PYOGENES

(Ogston 1881, Rosenbach 1884)

Source.—Abscess, cellulitis, erysipelas, meningitis, pneumonia, malignant endocarditis, puerperal endometritis, lymphangitis, otitis, periostitis, osteitis, osteomyelitis, etc. (Also *see* Pathogenicity.)

Morphology.—A minute dot-shaped organism usually arranged in chains, consisting of two or more organisms. The chains may be short or long, thus differentiating streptococcus brevis and streptococcus longus. The chains are best seen in the tissue exudates, or in pus, or from a liquid culture, as broth. The specimen should be handled very gently, otherwise the chains are liable to be dispersed.

Each organism measures 1μ in diameter. They are often classified as (*a*) streptococcus longus, consisting of long chains and are pathogenic, and (*b*) streptococcus brevis, consisting of short chains and are non-pathogenic. These are normally found in the mouth. They are non-motile, without flagella, with no spore and no capsule.

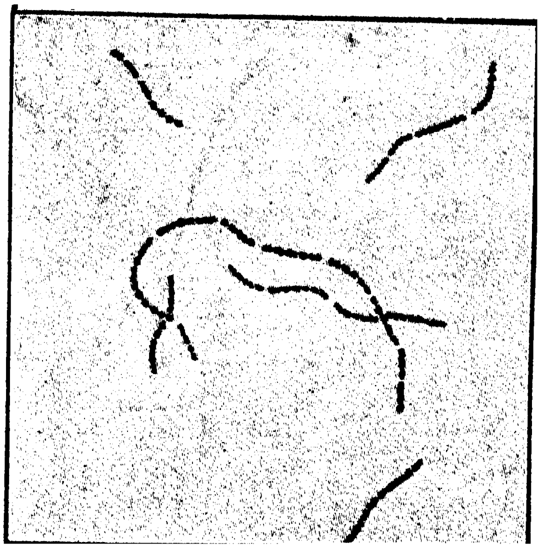


Fig. 39. Streptococcus.—Smear from broth culture.

Biology.—They grow best at 37°C., the extreme temperatures being 18°C. and 40°C. They are ærobes and facultative anærobes. Some forms of anærobic streptococcus were found in war wounds.

Staining Reaction.—Stains readily with all aniline dyes as well as by Gram.

Gram-negative Streptococci.—There are several species of streptococci which are Gram-negative. They are non-pathogenic.

Cultural Reaction and Biological Products.—Agar.—The colonies are very minute, like dots, and much smaller than those of staphylococcus. They are colourless, often looking like small drops of dew, and have got a tendency to remain discrete and separate. Broth—remains clear. Gelatin.—Same as in agar. Milk.—Coagulated by some varieties.

Streptococcus occupies a position intermediate between strictly parasitic forms (e.g., gonococcus and B. pertussis) which are difficult to cultivate and the enteric group which grow with greatest freedom on artificial media.

Variants.—Walker has observed the appearance of one or two colonies of mucoid appearance in plate cultures which far exceed the other strains in virulence and hæmolytic activity. Walker considers that these observations have a bearing on the serious epidemics of sore throat.

Fermentation of Sugar.—They attack a variety of sugars. Inulin is not fermented so as to differentiate from pneumococcus. For this test, Hiss's serum water medium containing inulin is used. Streptococcus is not soluble in bile.

Hæmolysis.—Some strains of streptococcus lead to hæmolysis of red blood corpuscles, while others do not. This can be tested as follows:—

(*a*) Inoculate the surface of a blood agar plate. Colonies are more luxuriant than on ordinary agar and are surrounded by a clear zone which indicates hæmolysis.

(*b*) Put ½ c.c. of blood into two citrated broth tubes. Inoculate one with streptococcus and keep the other as control. After incubation for 24 to 48 hours, the inoculated tube will turn pink on account of hæmolysis, and control will show the presence of corpuscles at the bottom.

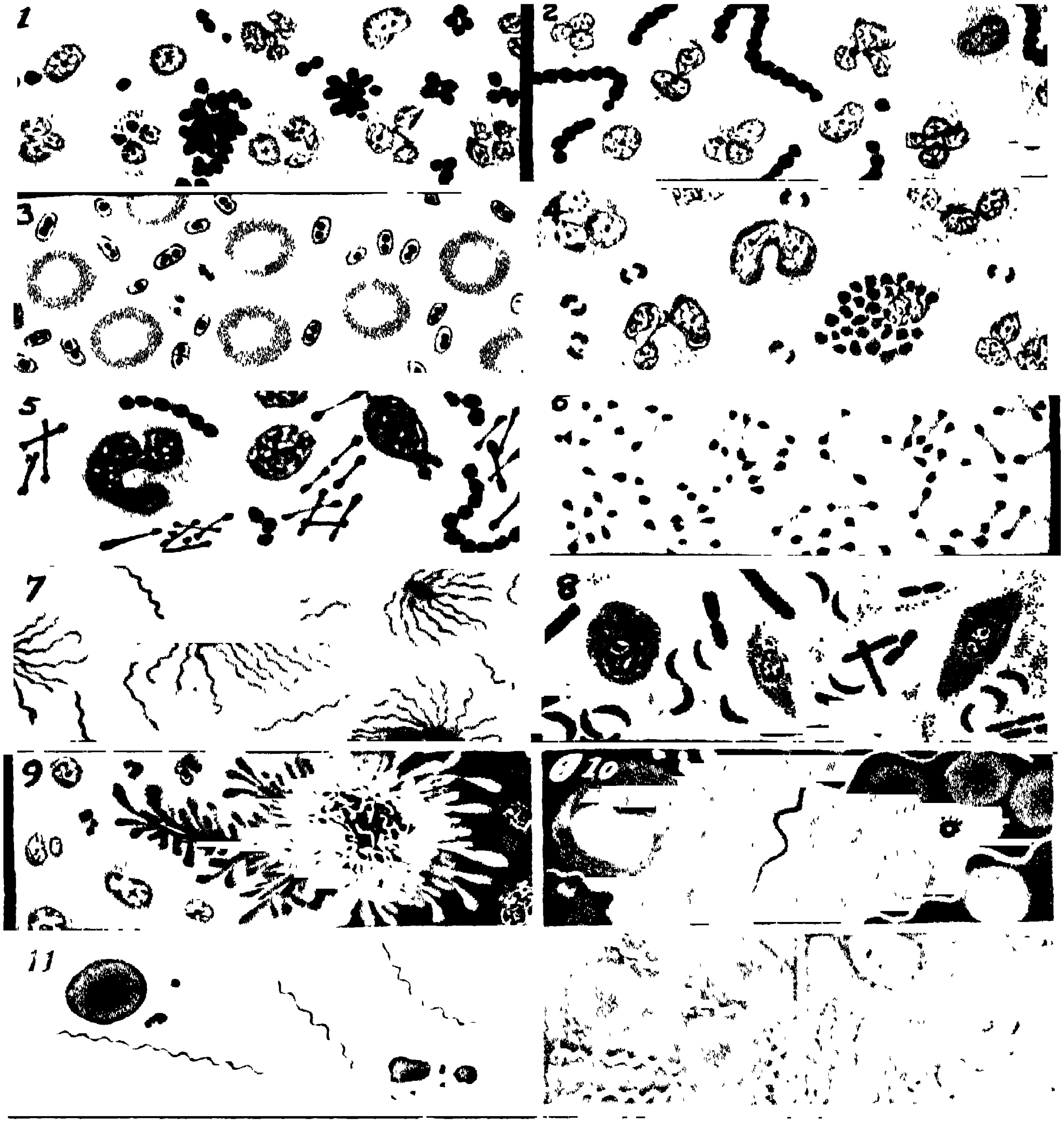
Brown differentiates the following types of hæmolysis.

1. α hæmolysis—a somewhat greenish discolouration and partial hæmolysis of blood corpuscles immediately surrounding the colony, and outside this a second clear non-discoloured zone. 2. β hæmolysis or true hæmolysis.—The colonies are surrounded by a sharply defined clear colourless zone of hæmolysis 3. α′ hæmolysis—The colonies are surrounded by a zone of hæmolysis which is slightly hazy containing a moderate number of altered corpuscles. The green colour is absent 4 No change on the surface but green discolouration in the depth of fresh blood agar plates.

Classification of Streptococcus.—Holman's classification based on hæmolysis and fermentation of lactose, mannite and salicin—is at present the best one. All streptococci ferment dextrose. Holman divides them into 8 hæmolytic and 8 non-hæmolytic types. The latter type has been further subdivided into (*a*) facultative ærobic or anærobic streptococci, or the mouth and bowel streptococci, and (*b*) strictly anærobic or non-pathogenic streptococci.

The terms "hæmolytic" and "viridans" are often applied to streptococci.

Plate No. 1.



MORPHOLOGICAL CHARACTERS OF SOME PATHOGENIC MICRO-ORGANISMS.

1. Staphylococcus pyogenes aureus (Gram's stain)
2 Streptococcus (Gram's stain)
3. Pneumococcus (Muir's Capsule stain.)
4. Gonococcus (Gram's stain).
5. Diphtheria Bacillus (Methylene Blue stain)
6. Diphtheria Bacillus (Neisser's stain)
7 B typhosus (Muir's Flagella stain)
8 V choleræ (Methylene Blue and Eosin stain)
9. Actinomyces bovis (Gram's stain)
10. S duttoni (Leishman's stain)
11 T pallidum as seen by the dark-ground illumination.
12. T pallidum and S refringens (Treated by Fontana's silver impregnation method).

Face Page 104

Inoculation Reaction.—This depends on the virulence of the organisms. In case of virulent organism the animal will die within 24 hours with enlargement of spleen and presence of the organism in the internal organs.

Pathogenicity to Human Body.—Streptococcus is responsible for the production of suppuration, erysipelas, septicæmia, pyæmia, malignant endocarditis, pericarditis, myocarditis, meningitis, arthritis, dermatitis, peurperal sepsis, osteomyelitis, etc.

The affection is more diffuse with streptococcal infection than with staphylococcus, and the response is greater. Streptococci are responsible for inflammatory and suppurative conditions varying greatly according to site, in severity and effects. They may also occur naturally as commensals.

Streptococci play a very important part in the causation of various affections produced by infection of different tissues of the body, *e.g.*,

(1) *Skin.*—(*i*) Erysipelas—True erysepelas is caused in most cases by hæmolytic streptococcus. Streptococci from a variety of sources have been made to produce erysepelas provided the virulence was sufficiently exalted by passage. Their multiplication in lymphatic spaces of cutis is attended by inflammatory œdema of the skin and adjoining tissues with infiltration of the spreading margin by mononuclear cells rather than by polymorphs as is usual in other streptococcal infections. (*ii*) Other affections.—Impetigo

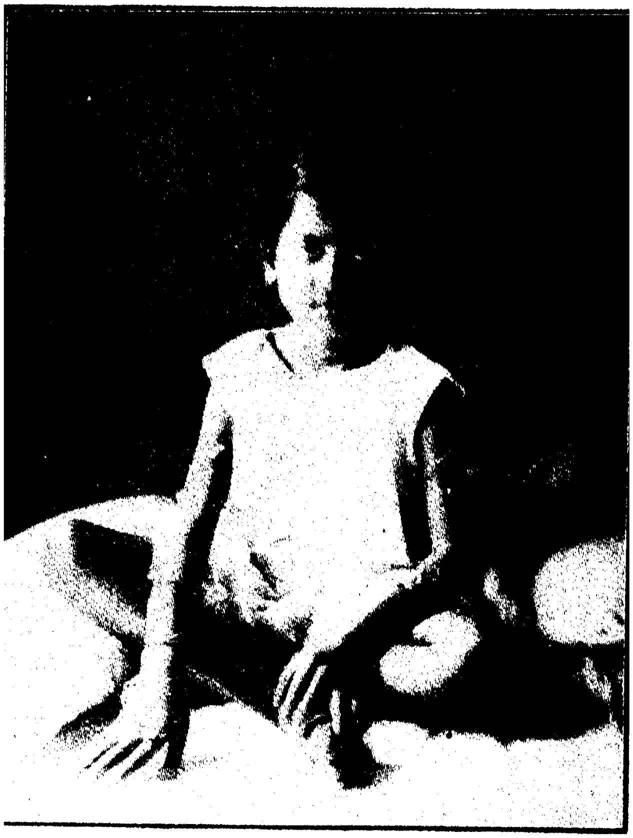


Fig. 40. Scarlet fever showing exfoliation of the surface of skin.
(Case of Dr. Bidhan Chandra Roy.)

contagiosum, paronychia and other such affections have been found to be due to varieties of streptococci.

(2) Subcutaneous tissue—Abscess, cellulitis, Ludwig's angina, wounds, lymphangitis.

(3) Bones and joints—Periostitis, acute suppurative arthritis.

(4) Respiratory passages—Otitis, broncho-pneumonia, tonsilitis, 'pharyngitis, etc. They are often present in the upper respiratory passages of healthy persons (about 36%).

(5) Scarlet fever (see below).

(6) Genito-urinary tract—Acute suppurative nephritis may follow scarlet fever, puerperal and other streptococcal affections. In puerperal sepsis, the role of streptococcus has been definitely established. The streptococci are generally transmitted to the genito-urinary tract through the hands of attendants, instruments etc., from streptococcal lesions *e.g.*, erysepelas, sore throat etc. either from the same patient or from other patients or even from attendants and nurses who are carriers of virulent organisms.

(7) Rheumatism and subacute endocarditis—The relation of these affections to streptococcus has not yet been definitely established

Streptococcus of Scarlet Fever.—Streptococci are always present in the throats of scarlet fever cases and also in the complications, *e.g.*, endocarditis, pericarditis, otitis media, etc. Dick and Dick have been able to produce the disease in man from streptococci isolated from scarlet fever cases.

Dick Test.—Blood agar slant cultures are diluted 1 in 1,000 in sterile salt solution and then filtered with Berkefeld filter. 0.1 c.c. of the filtrate is injected intracutaneously (the skin-test dose). In positive cases, an area of erythema, 1.5-3 cm. in diameter, appears in 1-4 hours reaching to the maximum in 18-36 hours, and then subsides in 48 hours. The Dicks found this reaction positive in over 40% of cases with no history of scarlet fever.

Scarlet Fever Antitoxin.—Antitoxic serum has been prepared from horse in which at least 1 c.c. neutralizes 1,000 skin-test doses.

Immunity.—

1. *Cells concerned in immunity.*—It has been observed that the presence of a large number of actively phagocytic leucocytes could not control streptococcal infections. Further, in immunised animals streptococci injected into the pleura could not invade the tissues, while in non-immunised animals they could invade widely before any leucocytic exudation could accumulate. These show that some other cells, *i.e.*, the histiocytes, commonly called the clasmatocytes, the reticulo-endothelial cells and the leucocytes, are responsible

for the arrest of extension of the streptococci. It has further been shown that exudates in which the clasmatocytes predominate, protect more efficiently than those containing polymorphonuclear leucocytes. Thus clasmatocytes are the elements in the immunity to streptococcus, although no appreciable bactericidal power can be demonstrated in clasmatocyte exudate *in vitro*.

2. *Antitoxin formation.*—The streptococcal toxin is capable of producing antiserum in immunised animals which will protect from streptococcal infection. This antiserum cannot neutralise the streptococcal product *in vivo* but renders the virulent streptococci susceptible for phagocytosis by leucocytes or cells of the reticulo-endothelial system. The nature of this process of immunity is one of neutralization of the toxin of the aggressin type.

Fæcal Streptococci.—These belong to two classes, the true streptococci and the enterococci. The enterococci are characterised by the presence of short chains often appearing as diplococci with a halo round them. These are generally mistaken for pneumococci, more so due to their lanceolate shape. These characters persist in broth culture, while in agar culture they appear more like staphylococci with no tendency of chain formation. These organisms are non-hæmolytic. Although they have repeatedly been found in appendicitis and abdominal sepsis, still it is believed that they play a very minor role in the pathogenicity for man.

PNEUMOCOCCUS (DIPLOCOCCUS PNEUMONIÆ)

(Frænkel 1884, Weichselbaum 1886)

Also known as diplococcus lanceolatus capsulatus extracellularis of Frænkel and Weichselbaum.

Source.—*See* Pathogenicity.

Morphology.—Minute dot-shaped organisms arranged in pairs, and hence known as *diplococcus.* The members of a pair are sometimes pointed only at the distal ends giving a lancet shape to which the organism owes its name *lanceolatus,* the flat ends being in apposition. The pair of organisms is surrounded by a well-marked capsule, giving the name *capsulatus.* The capsule appears as unstained halo and is lost in culture. This appearance is due to the refractive index of the capsule and of the media being equal. Thus, the disappearance of capsule in artificial culture is only apparent, as it can be demonstrated by special method with copper sulphate, the material from culture being diluted with serum. Each coccus is about 1μ in diameter, involution forms being not uncommon in old cultures. They are non-motile, with no flagella and without any spore. Pneumococcus often grows in chains, and, on account of the loss of capsule in culture, it requires differentiation from streptococcus. Its solubility in bile, property to ferment inulin, and failure to produce hæmolysis are the distinctive points.

Biology.—Optimum temperature 37.5°C., minimum 18°C, maximum 42°C. They are ærobes.

Staining Reaction.—Stains with aniline dyes as well as by Gram.

Cultural Reaction and Biological Products.—Agar, preferably Blood or glycerine agar—scanty growth of very fine dew-drop-like colonies which are clear and transparent like streptococcus. On Blood Agar the colonies are smooth, slightly raised, flat discs of variable size. Viewed by reflected light, they are colourless while by transmitted light they look more or less green (hæmolysis). Blood Broth.—Purple discolouration of blood which later becomes dark brown. This colour change is due to formation of methæmoglobin. The altered corpuscles are protected from hæmolysis as on blood plates.

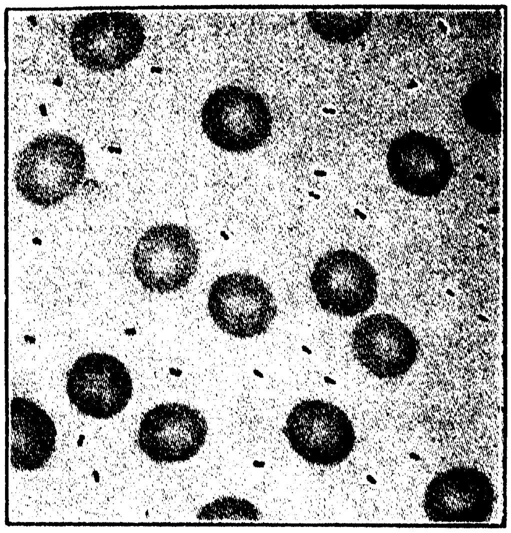


Fig. 41. Pneumococcus. Smear from blood.

Autolysis.—On further incubation after the maximum growth has occurred in broth the culture gradually becomes clear owing to autolysis. It is usual to find the broth culture after two days incubation quite clear as before the incubation

Other Media—Serum agar, fluid blood agar (human or animal), Hiss's inulin serum water.

Fermentation of Sugar. - For this purpose Hiss's serum water media containing inulin is suitable. Inulin is fermented by pneumococcus and the serum is coagulated Streptococcus does not act on inulin.

Litmus Milk.—Acid and coagulation on some occasions.

Bile Salts.—If sterile ox bile or sodium taurocholate is added to the culture-tube, the pneumococcus is dissolved (*cf.* streptococcus, which is not dissolved in bile). Pneumococcus killed by heat and avirulent pneumococcus are not dissolved.

A broth culture of pneumococcus when added to autoclaved bile (1 in 10), should be, after 10 minutes, as clear as the broth before inoculation. If with a dilution of 1 in 500 complete clearing does not occur after 1 hour at 37°C, the streptococcus in question should not be regarded as pneumococcus. The conditions necessary for the autolysis of pneumococcus and for bile solubility are identical. Therefore, bile salts help the natural autolytic process.

Intracellular products of Pneumococcus.—(1) A substance is found in the young culture of pneumococcus as well as in the tissue fluids of the patient *e.g.*, urine, serum, pleural exudates etc., which gives precipitin reaction with the homologous immune serum and thus serves as an additional method of diagnosis. This substance is a polysaccharide and is type-specific. (2) proteins, (3) autolysins, (4) hæmolysins (5) enzymes and (6) purpura producing substance.

Agglutination Reaction.—When tested with a high titre immune serum, there are found to be four different races of the organism, giving different reactions. From this agglutinating property, four types of pneumococcus have been differentiated. Type IV does not produce any group anti-serum.

Types I and II are the usual types present in most cases of pneumonia (mortality 30%). They are present in almost equal proportions in about 60% of cases. Type II is of more virulent form for man than Type I.

Type III is present in about 20% of cases and is the most virulent type. It possesses large capsule and forms viscid colonies in culture, and is hence also known as P. mucosus (mortality 50%).

Types IV is present in about 20% cases and is of the least virulence (mortality 12%).

Determination of the Type of Pneumococci from a Sample of Sputum

Things required—(1) Pure culture of pneumococcus; (2) Type sera of I, II, III from Rockefeller Institute, New York. Wash the sputum in sterile saline to remove surface contamination. Emulsify the washed sputum in saline and inject 0.5 c.c. of the same into a white mouse intraperitoneally. Kill the animal 8-10 hours after injection. Isolate the pneumococcus from heart's blood or peritoneal exudate in pure culture. Make an emulsion with saline. Dilute the Type sera of I, II, III as follows:—

Serum I—dilute 1 to 20. Serum II—dilute 1 to 20. Serum III—dilute 1 in 5. Take four tubes. Put in the first tube—0.5. c.c. emulsion of bacteria, add 0.5 c.c. of diluted serum I Put in the second tube—0.5 c.c. emulsion of bacteria, add 0.5 c.c. of diluted serum II. Put in the third tube—0.5 c.c. emulsion of bacteria, add 0.5 c.c. of diluted serum III. Put in the fourth tube—24 c.c. emulsion of bacteria, add 1 c.c. of sterile ox bile. Put them in water bath for ½ an hour at 37°C. Sedimentation in any of the first 3 tubes indicates the corresponding type. If no sedimentation occurs, the organism belongs to the Type IV, all the strains will be soluble in bile, as indicated by the clearing up of the turbidity of the culture.

Inoculation Reaction. –Pneumococcus is extremely pathogenic to mice, less in rabbits, and least in guinea-pigs. Pigeons are immune. In mice, septicæmia followed by death occurs in 24 hours. Culture from heart's blood gives pure growth.

Capsule is present when the organism is examined from human tissue. This is lost in culture. When an animal is inoculated with this culture, the blood and the tissue of the dead animal will again show the presence of capsule surrounding the organism.

While pneumococcus is pathogenic to many animals, the lesions found do not resemble true lobar pneumonia of man, even when introduced by tracheal, pleural or intra-pulmonary injections, except in very few occasions.

Rôle of Pneumococcus in Man.—Pneumococcus is present in the mouths and throat of at least 20% of healthy individuals. The most frequent condition produced by pneumococcus is a catarrhal condition of the upper respiratory tract often popularly described as influenza. Lobar pneumonia is produced. The genesis of its development is explained by assuming that the virulent pneumococci gaining access to the trachea or bronchi penetrate deeply into the mucous membrane and invade the peribronchial tissue and thence extend to the adjacent lung tissue. Some degree of septicæmia takes place simultaneously.

Pathogenicity to Man.—Causes lobar pneumonia (80% cases), broncho-pneumonia, pericarditis, peritonitis, empyema, meningitis, endocarditis, arthritis, otitis media, etc.

Pneumonia is a form of septicæmia with intense toxæmia. In a large proportion of lobar pneumonia, pneumococcus can be cultivated from the blood of the patients. The term pneumonia is very indefinite. The so called pneumonia, although commonly caused by pneumococcus, may also be caused by other organisms, *e.g.,* B. influenza, B. pestis, B. tuberculosis, streptococcus, Pneumobacillus, etc.

Pneumococci, on the other hand, are found not only in most instances of lobar pneumonia, but also in bronchopneumonia and secondary pneumonia; in the latter condition, they are associated with other organisms *e.g.,* B. diphtheria, B. influenza or streptococcus.

Pneumococci may occasionally be found in the throat and mouth of healthy persons. They usually belong to Type IV, which is the least virulent form for the human beings. They usually become pathogenic when the soil becomes ready for their growth, *e.g.*, after an exposure to cold, an attack of bronchitis or an excess of alcohol.

Variation of the Lesion Caused by Different Types.—Pneumonia due to Type I pneumococci is complicated twice as frequently with empyema. That due to Type III shows a moist viscid exudate.

Therapeutic Use of Pneumococcal Serum.—A strictly type-specific antiserum has been prepared. Antiserum of one type has no action on infection with another type, except the non-specific effect due to normal horse-serum. Antibodies from different monovalent sera may be combined, but a curative polyvalent serum has not yet been prepared. An antiserum is of curative value in case of the organism belonging to Type I, less so with Type II and has no effect in cases due to Type III. In case of Type I, again, it requires a massive dose of the antiserum, say about 100 c.c. each time, given intravenously and repeated every eight hours for two or three times.

MICROCOCCUS TETRAGENA

(Gaffky 1881)

Source.—Found originally in the sputum and contents of a phthisical cavity. Found generally in the respiratory tract, and, occasionally, in suppuration.

Morphology.—Minute dots, arranged in " tetrads " or in groups of four. Size, about 1μ in diameter. They are non-motile, possess no spore and no flagella, and are surrounded by a capsule.

Biology.—Grow well in ordinary temperature, optimum being 37.5°C. They are ærobes.

Staining Reaction.—Readily stained with aniline dyes and are Gram-positive.

Cultural Reactions.—AGAR,—moist, sticky, shining, greyish-white cream-coloured growth. BROTH.—turbidity.

Inoculation Reaction.—Inoculation into a mouse or guinea-pig leads to production of a local suppuration, and, occasionally, septicæmia. The organisms are found in blood, spleen and other organs.

GONOCOCCUS (NEISSERIA GONORRHŒA)

(Neisser 1879)

Source.—Found in the discharges from the genito-urinary tract in case of gonorrhœa, and from the conjunctiva in ophthalmia.

Morphology.—Minute dots, 1μ in diameter, arranged in pairs (hence called *diplococcus*); each individual coccus is more or less bean-shaped, with the concave surfaces close together. The organisms are non-motile, and possess no capsule, no spore and no flagella. They are usually found within the leucocytes forming the pus cells and adhering to the surface of epithelial cells present in the pus without injury to their morphological and biological properties. They are also found lying free in the discharge.

Biology.—They grow at 37°C. and are ærobes. The viability of gonococci is very low. They are very susceptible to desiccation and rise of temperature. Cultures exposed to 40°C. for a few hours usually lead to death of the organisms but not to those in the tissues. A temperature of 107°F. (42°C.), as given by diathermy, kills the organisms in the urethra in about an hour.

Staining Reaction.—They are readily stained with the common aniline dyes but do not stain by Gram's method.

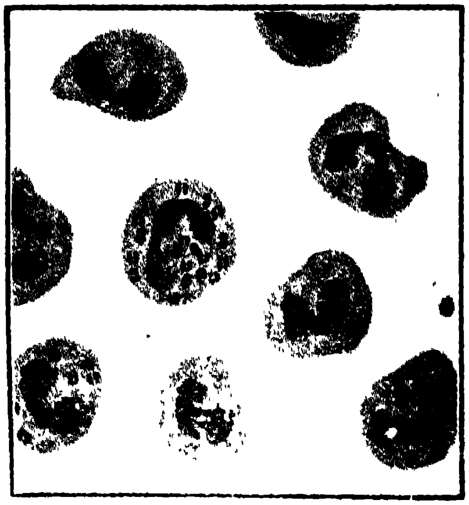


Fig. 42. Gonococcus.– Smear from urethral discharge.

Cultural Reactions.—AGAR and BROTH not suitable for cultivation. These organisms do not grow on the ordinary culture media, unless special methods are adopted. They grow when some amount of uncoagulated body protein is present in the media. For the same reason, serum, whole blood; ascitic fluid, hydrocele or pleural fluid, has been used. Instead of human body fluid, beef serum, rabbit serum, etc. have also been substituted.

It was very often found that the growth of the organism is abundant if it is allowed to grow in the presence of low oxygen tension of the atmosphere. Bose adopted a method very suitable for primary culture. This consists in planting out the infected material on whole blood agar and incubating it in an atmosphere of carbon dioxide.

Gonococcus undergoes rapid autolysis in culture. Even a 24-hours' growth at 37°C shows degenerated organism showing involution forms. Giant forms, several times the size of normal cocci, are often met with. The degenerated cocci are found more at the centre of the colony while the normal ones are found at the margin.

Necessary conditions for the best growth of gonococci. TEMPERATURE.—Primary growths are best obtained at 35° to 36°C although some strains grow between 26-39°C. Not only the growth is more rapid and luxurient at 35°C than at 37°C, but cultures at lower temperature do not undergo autolysis to the same extent as when inoculated at high temperature. MOISTURE.—Gonococcus is remarkably susceptible to variation in moisture of the surface on which it grows and the atmosphere in which it is incubated. The mean optimum reaction for growth is pH 7.6.

Polar bodies.—Gonococcus when cultivated in medium rich in animal nucleo-proteins develop polar bodies which appear as metachromatic granules attached to gonococcus. They are thought to be of special value in the preparation of vaccines.

Serological reactions.—1. Complement fixation.—Positive reaction is of signification while the negative is not, 2. Agglutination—Not certain.

Agglutination Reaction.—Gonococcus is agglutinated by the antiserum, but its specificity is not settled, as some strains of meningococci are also agglutinated by anti-gonococcal serum.

By means of absorption ot agglutinins several types of gonococci have been differentiated, of which there is a predominant type constituting about 70% of all strains responsible for gonorrhœa.

Inoculation Reactions.—Not satisfactory, even in anthropoid apes, through urethra. By intraperitoneal inoculation of white mice, the organisms can be recovered from the peritoneal exudate or, rarely, from the heart's blood.

Pathogenicity to Man.—It causes inflammation of the mucous membrane of the urethra and spreads upwards and affects the genito-urinary tract. Bubo, septicæmia, malignant endocarditis, meningitis, arthritis, ophthalmia, etc., are also caused by this organism.

The organism first affects the anterior part of the urethra, and then the posterior part, bladder, ureter, kidneys up to the perinephric tissue on one hand, and every individual part of the genital tract, on the other, both in the male and female. The blood is also affected, which carries the organisms to distant parts, causing general manifestations, *e.g.*, septicæmia, or local lesions, *e.g.*, arthritis, endocarditis, etc. Bubo is the direct extension of infection through the lymphatics, and ophthalmia, the result of mechanical inoculation by finger, etc.

The portals of entry of gonococcus into human subject are two in number—through the genito-urinary tract and the conjunctival mucosa. The earlier stages of infection are characterised by a catarrhal inflammation proceeding later to pus formation. Smears of material taken on the third day of illness exhibit the presence of a layer of pus on the mucosa containing numerous gonococci within and between pus cells. Gonococci penetrate very little in areas covered with squamous epithelium with some amount of desquamation of the same. Where the surface is covered by cylindrical epithelium, there is evident intracellular penetration of the cocci and the inflammatory changes are most conspicuous around the mucous glands where gonococci can be seen in large numbers. They invade the submucosa resulting in round celled infiltration of the tissues.

The condition may undergo almost complete resolution but in large number of cases a sub-acute or chronic inflammatory condition remains with gonococci situated mainly in the glands. If the process be of severe type occurring over a fairly extensive area of urethral mucosa, healing takes place by formation of fibrous tissue, the contraction of which leads to urethral stricture.

Secondary Infections in Gonorrhœa.—In all cases, specially in chronic ones, a good number of other organisms are present. Mischievously vigorous treatment may be partly responsible for the same. Other Gram-negative diplococci, *i.e.*, M. catarrhalis or Gram-positive cocci which have lost their Gram-positive quality by being phagocytosed, may be mistaken for gonococci.

Provocative Methods of Diagnosis.—In chronic cases where the demonstration of organisms is extremely difficult, they can be demonstrated by means of provocative methods. These fall under two groups: (*a*) *Non-specific.*—Local application of silver nitrate which leads to irritation and flow of fluid, may bring the organism superficially from a deep-seated latent infection. (*b*) *Specific.*—Subcutaneous injection of gonococcal vaccine, in a dose of 200-500 million killed cocci produces definite focal and general reaction in gonorrhœal cases but not in normal persons.

The general blood infection is in the form of pyæmia (gonorrhœal arthritis, synovitis, etc.) or rarely septicæmia (endocarditis).

MENINGOCOCCUS (NEISSERIA INTRACELLULARIS)
(Weichselbaum 1887)

(Also called *Diplococcus meningitidis intracellularis.*)

Source.—It occurs in the cerebro-spinal fluid, blood, upper respiratory passage, nose, eye, ear and joints.

Morphology.—In the tissue they are minute cocci, about 1 μ in diameter, arranged in pairs or singly inside a polymorphonuclear leucocyte. They are non-motile, with no flagella, no spore and no capsule. Involution forms common in old culture. They are delicate parasites often killed by drying for one day only. They often die in the culture medium itself if kept for a few days.

Biology.—Grow best at body temperature. They are ærobes.

Staining Reactions.—Stain readily with ordinary aniline dyes. They are Gram-negative and stain well with Leishman.

Cultural Reactions.—AGAR and BROTH—the organism does not grow in primary culture. Subculture grows well. GLYCERINE AGAR—grows well. NASGAR (neutrose ascitic agar)—grows best. TRYPAGAR (agar with ascitic fluid, blood or serum)—grows well. The colonies are moist, grey, oval or circular, translucent and with regular margin. GELATIN—grows well, with liquefaction.

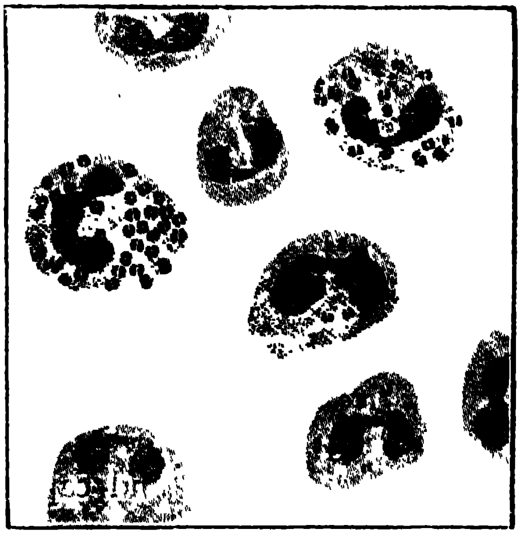


Fig. 43. Meningococcus—Smear from cerebro-spinal fluid.

Fermentation of Sugars.—Some sugars, *e.g.*, glucose, maltose, etc., are fermented with production of acid. Lactose, lævulose, galactose, etc., are not fermented.

Differentiation.—(1) From M. catarrhalis:—by the property of meningococcus to ferment dextrose and maltose without producing gas

(2) From Gonococcus:—

Meningococcus:—	Gonococcus:—
Grows well on ordinary media especially in subculture.	Does not grow on ordinary media.
Abundant growth in serum	Very scanty growth in serum
Ferments glucose and maltose.	Ferments glucose only.

Agglutination.—Some strains give agglutination reaction but not always. Gordon recognised four types according to the agglutination reactions.

Inoculation Reaction.—Mice and guinea-pigs are susceptible when injected intraperitoneally but not subcutaneously. Monkey gets typical meningitis when inoculated intraspinally.

Pathogenicity.—It causes sporadic and epidemic cerebro-spinal meningitis, and can be obtained by lumbar puncture. The organisms for posterior basic meningitis are almost identical to meningococcus. They have also been found to occur in arthritis, pneumonia, pericarditis and other complications during the course of meningitis. They are found rarely in blood. They are often found normally in the naso-pharynx of the patients suffering from the disease or those who come in close contact with a patient—the so-called *carriers.*

Antimeningococcic Serum.—This has been introduced by Flexner. This is usually used therapeutically by injecting into the spinal canal by means of lumbar puncture.

MICROCOCCUS CATARRHALIS

(Seifert 1890)

Source.—Occasionally present in the sputum of healthy persons, found in nasal and bronchial secretions, conjunctiva, etc.

Morphology—It is a diplococcus, occurring in pairs, often inside a polymorphonuclear leucocyte. Motility, flagella, spore and capsule are all absent.

Biology.—Grows at body temperature, and is an ærobe.

Staining Reaction.—Stains well with aniline dyes, and is Gram-negative.

Cultural Reactions.—AGAR and BROTH—grows well. BLOOD or NASGAR medium (*see* meningococcus) are suitable. The colonies are white, moist and translucent.

MICROCOCCUS MELITENSIS (BRUCELLA MELITENSIS)

(Bruce 1887)

Source—Blood, spleen or urine from a case of Malta fever. Also known as Mediterranean fever or undulant fever. In nature, found in dust, clothing and water.

Morphology.—The organism, a minute coccus, is the smallest organism known, being 0.4μ in diameter. In culture it often appears as a distinct bacillus. It occurs in clumps, or in pairs, or in short chains There is no motility, no flagella, no spore, no capsule. Gordon described the presence of motility and flagella, but was not corroborated by subsequent observers

Biology.—Grows well at blood heat, is killed at 55°C. and is an ærobe.

Staining Reactions.—It stains well with aniline stains but is Gram-negative.

Cultural Reactions and Biological Products.—AGAR.—Minute transparent colonies appearing in 3 to 6 days. Old cultures are opaque. BROTH.—Slight turbidity. LITMUS MILK.—Alkaline without coagulation.

Agglutination Reaction.—Serum of patients suffering from Malta fever agglutinates the emulsion of M. melitensis.

Inoculation Reaction.—Guinea-pig and rabbit are not susceptible until inoculated intra cerebrally. Monkeys are susceptible, getting fever and enlargement of spleen, and, occasionally, death.

Pathogenicity.—Causes Malta fever. Usually transmitted through goat's milk. The organisms are not destroyed by the souring of milk; hence butter and cheese from infected milk may also transmit the disease.

CHAPTER X

BACILLI

ACID-FAST ORGANISMS

The organisms under this group possess the property of retaining the stain of fuchsin when treated subsequently with sulphuric or any mineral acid. This property is due to the presence of a fatty substance in the organisms which resists the decolourisation.

B. TUBERCULOSIS (MYCOBACTERIUM TUBERCULOSIS)

(Koch 1882)

Source.—Sputum and fæces, in cases of pulmonary and alimentary tuberculosis, and tissues, in cases of tuberculosis of the organs or glands. Dust and other materials are contaminated by the sputum or excreta of patients suffering from pulmonary or alimentary tuberculosis.

Morphology.—The organism is a slender rod, measuring 2-5 μ in length and 0.5 in breadth. Occasionally, in sputum and other sources, the organisms show a beaded appearance, due to the presence of some unstained spaces in the organism. This appearance is not constant and, occasionally, all grades of the beads are present in the same sample of material.

The beaded appearance is seen more distinctly when stained by Gram's method than by the Ziehl-Neelsen method. It is now usually believed that these granules, giving the beaded appearance, represent reserve material of special chemical composition and they must not be mistaken for spores, as B. tuberculosis forms no spore and the granules possess no special resistance to heat or disinfectants. It usually remains as a single individual, occasionally in pairs, often arranged like the letter V and, rarely, in clumps. In old cultures, involution forms occur and, not infrequently, form branches.

Tubercle bacillus is non motile; there is no flagella, no spore and no capsule.

Biology.—The tubercle bacillus is ærobic and is a facultative anærobe and grows at 37.5°C. as the optimum temperature. The organisms possess great powers of resistance to external agencies, probably through the fatty envelope. They can live in dried sputum for more than two months. They also resist putrefaction for a considerable time, if kept in distilled water or putrefied sputum or tuberculous tissue. They can resist considerable heat (about 70°C.) and also exposure to gastric secretion for 6 hours.

They are rapidly killed if exposed to direct sunlight and also by strong antiseptics, *e.g.*, carbolic acid.

Staining Reaction.—The tubercle bacillus stains with some difficulty with the ordinary aniline dyes. It stains well with Gram's method.

Special Stain.—ZIEHL-NEELSEN METHOD.—By this stain, the tubercle bacilli, along with all the other organisms and tissue elements, are stained deeply with carbol fuchsin. When this is treated with sulphuric acid (20%), and also with alcohol, the colour is discharged from all the organisms and tissue elements except the tubercle bacillus. Due to the presence of this property, the bacillus is called *acid-fast* and *alcohol-fast*, the acid-fastness being due to the presence of certain chemical substances (*lipoids*) in the organism. Counterstaining with methylene blue, the materials decolourised by the acid are stained. Thus, in a film stained with this method, the tubercle bacilli are stained red, whereas the other organisms are stained blue. The colour of fuchsin from the tubercle bacilli is not removed by washing even in acid-alcohol.

Fig. 44. Tubercle bacillus in culture.

Cultural Reactions —The tubercle bacillus is difficult to culture, ordinary media being absolutely useless for its cultivation. Even in the special media, it takes at least 3-6 weeks to have an appreciable growth. The special media for primary cultures are Dorset's egg medium or glycerinated potato. Those for subcultures are coagulated bovine serum and other glycerinated media, *e.g.*, glycerine agar, glycerine broth, glycerine gelatin, glycerine potato, glycerine brain agar, glycerine egg (the most convenient medium), and Petroff's medium. This last contains gentian violet which inhibits the growth of other organisms.

The appearance of the growth in solid media is of a dry, dull, wrinkled, cream-coloured character. The growth consists of a compact mass which comes off as a scale when lifted up by the needle.

It can be smeared on the slide only with great difficulty on account of the compactness of the growth.

In a liquid medium, it appears as a soft, dry, cream-coloured, wrinkled film floating on the surface of the medium, occasionally spreading and filling up the whole surface. It does not liquefy gelatin.

On EGG, colonies of human bacilli are whitish centrally and more raised than bovine types. The central part grows out to form opaque creamy white mass. On BOVINE SERUM, cultures of the human type of organism exhibit a yellow pigment. On GLYCERINE MEDIA, the human bacillus is " Eugonic," *i.e.*, it grows luxuriantly, producing thick, creamy, wrinkled or warty colonies.

The bovine type.—In primary culture on EGG, two to three weeks old colonies are small, flat and transparent with slightly raised centres. Generally the growth of bovine bacillus is inhibited by glycerine and only few bacilli succeed in forming colonies.

The Avian type.—On EGG, the colonies become visible to the naked eye on the 9th to the 12th day as minute points. On GLYCERINE EGG the colonies are visible a little sooner than on egg and they grow to a larger size.

Bacillus tuberculosis of cold blooded Animals.—Acid-fast bacilli have been isolated from fish, frogs, turtles, snakes, aligators etc. The bacilli are ærobic and grow best at 25°C The strains grow on the surface of broth producing pellicles of varying thickness. The growth is favoured by the addition of glycerine and glucose to the media.

Agglutination Reaction.—The tubercle bacillus is agglutinated by the blood serum of man or animal suffering from tuberculosis.

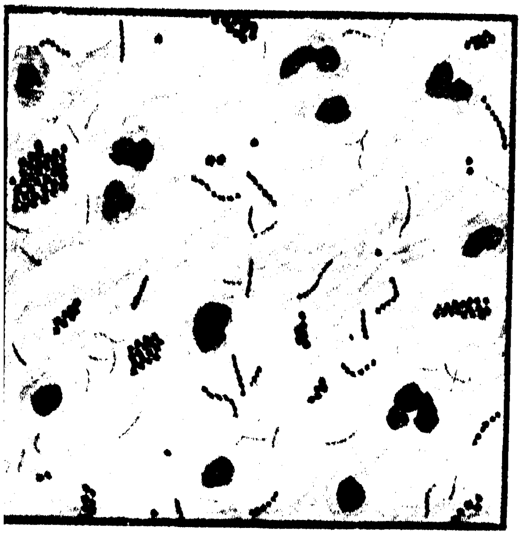
Inoculation Reaction.—The guinea-pig is the most susceptible animal. The animal is injected subcutaneously. The adjacent lymph nodes become enlarged, the animal loses weight and it leads to generalised tuberculosis in a period of about 3 to 6 weeks, with subsequent death. Spleen is enormously enlarged and studded with numerous tubercles.

Pathogenesis.– Man is very frequently infected. The tubercle bacillus always infects the lymphatic tissues mainly, and hence, leads to tuberculosis of lungs, peritonitis, meningitis, pericarditis, pleurisy, etc., subsequently affecting the glands, intestines, bones, etc.

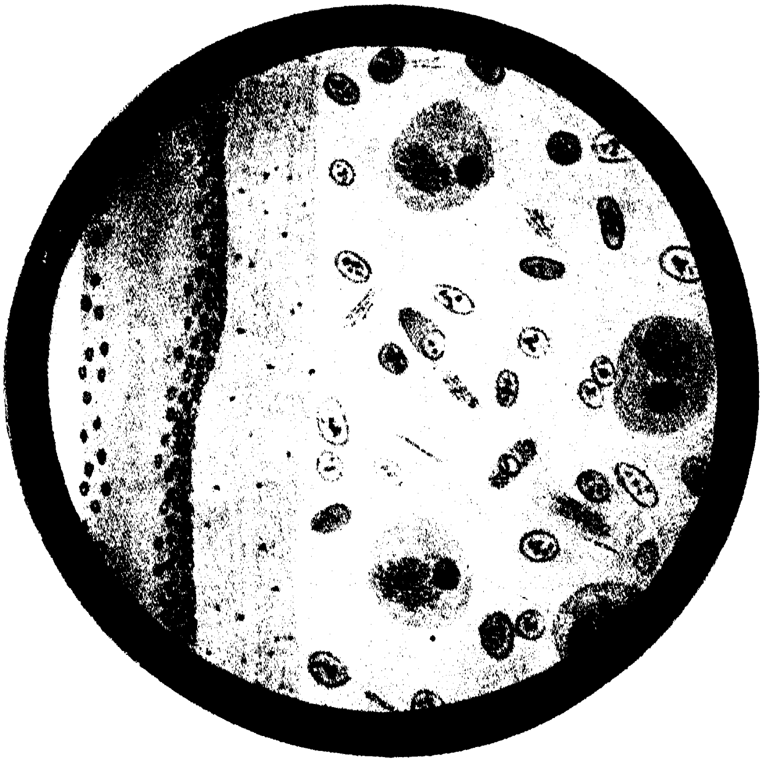
The genito-urinary tract is very often affected by tuberculosis Lupus is true tuberculosis of the skin.

Large number of domestic animals, wild animals under captivity, birds are subject to tuberculosis like man; cattle, horse and pig are the commonest animals to suffer.

Plate No. 2



Tubercle Bacillus in Sputum
(Ziehl-Neelsen Stain).



Lepra Bacillus in Section of Skin,
(Ziehl-Neelsen stain).

Face page 120

Although man and cattle suffer from the same kinds of infection with the tubercle bacillus, and, occasionally, man is infected from the bacillus of bovine origin, still, there is some ***difference between the two types of the tubercule bacillus.*** They are as follows:—

	Human	Bovine
Occurrence	Pulmonary infection.	Infection of glands, joints.
Morphology	Longer, thinner.	Shorter, thicker.
Culture	Easier.	Difficult.
Inoculation in rabbit.	Produces small discrete lesions. Animal lives for more than three months.	Large caseating masses. Animal dies within five weeks.
Cattle	Refractory.	Sensitive
Virulence	More for human than for animals	More for animals than for man.

Distribution of the Organisms in Tissue.—They are present in small number in chronic lesions, much less when the lesion becomes caseous. In subacute lesions the organisms are few in number, but in acute lesions they are very numerous. In the tubercle itself, they are mostly present near about the centre. The gaint cells found in the tubercle often contain a fair number of the organisms, mostly found at the periphery of the giant cell, like the circumferential arrangement of the nuclei characteristic of these cells.

The organisms pass out of the body through the discharges from the tuberculous focus, or through the secretions of the infected organs, *e.g.*, sputum, urine, cerebro-spinal fluid, etc.

In chronic tuberculous lesions one often finds no acid-fast organisms, but the material is found to be infective to guinea pig.

Much (1908), by a modified Gram's method of staining, found in these acid-fast bacilli isolated or clumped granular material which is believed by him to represent a virulent non-acid-fast form of tubercle bacillus. These granules are called after him, *Much's granules.*

Calmette and others claim to have found the existence of one ultramicroscopic filtrable virus in tuberculosis. This has not been corroborated by many.

Tuberculin

Tuberculins are the preparations from suspensions or extracts of tubercle bacillus.

Tubercle bacillus has got no exotoxin, but when the organism is cultivated in glycerine broth, a substance is found in the medium which is toxic (old tuberculin) and yields a proteose and an alkaloid on chemical examination. The toxic substances are derived usually from the grown-up bacilli themselves.

The following are the common forms of tuberculin used:—

1. *Old Tuberculin.*—The tubercle bacilli are grown on glycerine broth kept for several weeks in the presence of a free supply of air. The growth of the organism is killed in the autoclave and passed through Pasteur-Chamberland porcelain filter. The filtrate is the tuberculin and is known as *Koch's Old Tuberculin.* It consists of the products of the bacteria produced in the culture medium along with the medium itself.

2. *Tuberculin O. (also known as " T. O.").*—The organisms are grown on the surface of solid medium. The growth is taken off and is dried *in vacuo.* This dried mass is then ground up in an agate mortar, which is then emulsified and centrifugalised. The clear supernatant fluid is decanted off, and constitutes the T. O. The product is not precipitated by glycerine.

3. *Tuberculin R. (also known as " T. R.").*—After the supernatant fluid, T. O., has been decanted off, the residue is again ground in the mortar with distilled water. It is then centrifugalised and the supernatant fluid decanted off. The sediment is again ground, emulsified, and centrifugalised. The clear fluid is decanted and mixed with the former quantity. The sediment is again treated in a similar way, until there is no residue left behind. All the decanted fluids are mixed together. This constitutes T. R., and is precipitated by glycerine.

4. *New Tuberculin (also known as " Bazillen-emulsion ").*—The bacterial mass is dried, ground, emulsified and centrifugalised, until no sediment forms. The total amount of clear fluid, after each decantation, forms the tuberculin. Hence, *New Tuberculin is a mixture of T. O. and T. R.* 1 c.c. of this preparation contains 5 mgm. of bacillary substance.

5. The " *Filtered Bouillon* " *of Denys* is a filtrate from 5% glycerine peptone broth cultures of the bacillus with 0.25% phenol as preservative. This corresponds to Koch's Old Tuberculin, only it is unheated.

Tuberculin Reactions.—*Vide* under diagnosis. (p. 125).

Immunity Reaction.—*Vide* under examination of blood (opsonic index).

6. B. C. G. Vaccine (Bacillus Calmette-Guerin vaccine). Calmette and Guerin have advocated a vaccine of a living culture attenuated by growth in bile as a method of establishing immunity in newly born offspring of tuberculous parents.

Experimental tuberculosis

The effects of experimental tuberculosis in an animal depend on several factors (1) the virulence of the strain, (2) the number of living bacilli introduced, (3) the mode of infection and (4) the susceptibility of the animal.

Monkeys and guinea pigs are very susceptible to the mammalian tubercle bacilli, rabbits to bovine and fowls to avian bacilli. The disease is acute or chronic according to the number of bacilli introduced. In the calf, small doses of virulent bovine bacilli inoculated subcutaneously or given with food often produce only limited localised lesions, which become retrogressive. Medium-sized doses (10 mgm. subcutaneously) give irregular results, some calves dying of fatal general tuberculosis others serviving and showing different degrees of general progressive and retrogressive tuberculosis. Large doses (50 mgm. subcutaneously) invariably give rise to general tuberculosis which is fatal within three months. Much larger doses are required to produce rapidly fatal general progressive tuberculosis in calves by feeding than by subcutaneous inoculation. On the other hand, extremely small doses suffice to cause rapidly fatal general progressive tuberculosis when inoculated intravenously.

Distribution of lesions.—*Intravenous inoculation* of virulent bacilli always produces a very severe type of tuberculosis in susceptible animals—the acute septicæmic type first described by Yersin. The organisms are distributed to various organs where they settle and multiply. Generalised miliary tuberculosis is produced with the lungs as the seat of greatest severity. In non-susceptible animals, chronic form of tuberculosis is produced.

Intraperitoneal inoculation produces a less acute process. The organisms are partly distributed through the general circulation and partly retained in the omentum which becomes a mass of tuberculous caseating tissue adhering to surrounding structures. The peritoneum becomes studded with tubercles. *Subcutaneous inoculation* produces a lesion in which the organisms, for the most part, remain lodged in the subcutaneous tissues where they give rise to a caseo-necrotic tumour varying greatly according to the number of the organisms. The bacilli then begin to invade the adjacent lymph glands which enlarge progressively and become wholly or in part converted into caseous masses. If the dose is not large, in addition to miliary tuberculosis, masses and nodules of varying size are seen. *Inhalation* of air containing tubercle bacilli readily produces pulmonary tuberculosis in susceptible animals. *Feeding* is not always followed by formation of tubercles. The ingested organisms may either pass straight through the alimentary canal, or if they penetrate the mucous membrane, be taken up by phagocytes and destroyed. When the bacilli escape immediate destruction, they either remain in the mucous membrane and set up characteristic lesions forming the tuberculous ulcers or they pass through the mucous membrane into the nearest lymphatic glands, leaving no trace of their passage. In the glands, again, great majority of the bacilli are arrested, but some are carried by the lymph and blood stream to the lungs and even beyond. *Occular instillation* produces typical cervical adenitis accompanied with inflammatory changes in the eye and nose.

The most important point brought out by the inoculation tests is that at whatever point the tuberculous virus is introduced or penetrates an epithelial surface, there is in the adjacent lymphatic glands a tuberculous lesion appearing.

Laboratory Diagnosis of Tuberculosis

A. Isolation of Tubercle Bacillus by Culture

(*i*) From tuberculous materials not contaminated by other organisms, *e.g.*, tuberculous gland, etc.

(*a*) Macerate the gland with sterile water or saline in a sterile vessel. Inoculate four or five Dorset's egg media with the material. Take great care to avoid contamination, see that there are a few drops of water at the bottom of the tube to supply moisture. If there be none, put a few drops of sterile water. After inoculation, seal the mouth of the tube, to prevent evaporation and contamination, by means of paraffin or a rubber cap. Incubate at 37.5°C, and examine on the 8th day for the colonies. Colonies become visible at the end of the first week, and become distinct by the end of the second week.

(*b*) Another method—*Inoscopy*.—Digest the gland with pepsin-hydrochloric acid, centrifugalise and inoculate.

(*c*) The gland or other tissue is frozen as in cutting section with freezing microtome. Fine sections are cut which are treated with antiformin, as in the case of sputum, etc., as described below.

(*ii*) From tuberculous materials contaminated by other organisms, *e.g.*, sputum, fæces, pus, urine etc.

By the *Antiformin Method*.—Antiformin is a combination of equal parts of liq. sodæ chlorinatæ and 15% solution of caustic soda or potash. Caustic soda or potash serves the purpose of dissolving the mucus of the sputum, rendering it easy for centrifugalising and sedimenting the organisms. Liq. sodæ chlorinatæ has got the property of killing all other organisms except tubercle bacilli. Caustic soda or potash (5% solution) may often be substituted for antiformin.

Equal parts of sputum containing tubercle bacillus and antiformin are mixed thoroughly in a sterile centrifuge tube, and kept at room temperature for 2-3 hours, so as to dissolve the mucus. The tube is then centrifugalised, the supernatant fluid is decanted, and the tube again filled up with sterile distilled water. This is again centrifugalised, the supernatant fluid decanted, and the whole process repeated 3 or 4 times. The sediment is then used for inoculating the culture media. For diagnosis the sediment can be divided into three portions, and used as follows:—

(*a*) One portion used for immediate examination by staining with Ziehl-Neelsen process.
(*b*) Second portion used for culturing in Dorset's egg medium.
(*c*) Third portion used for inoculating a guinea-pig.

B. Phenomenon of Hypersensitiveness—Tuberculin reactions

1. *Von Pirquet test.*—The skin over the flexor surface of the fore-arm is cleansed and two drops of 50 and 25% tuberculin diluted with glycerine is placed and the epidermis is scarified with a sharp needle without drawing blood. The tuberculin is allowed to act for ten minutes then wiped off. Scarification is also made through a control drop of glycerine, midway between the two drops of tuberculin. With a positive reaction an inflammatory redness and swelling appear round the site of inoculations developing within 24 hours with a papule half an inch in diamater and reaching its maximum within 48 hours, and then gradually receeding. A negative test is of great value especially in children.

2. *Moro's test*—This consists in rubbing into the skin for about a minute a paste made with equal parts of old tuberculin and lanolin Reaction is evidenced by the appearance of papules in 24 hours.

3. *Ophthalmoreaction of Calmette*—1% of old tuberculin is instilled into the conjunctiva. This test is now discarded owing to disagreeably severe reaction, often amounting to loss of the eye.

4. *Subcutaneous inoculation of* 1|5 mgm. of old tuberculin.

C. Demonstration of Immune Bodies

1. *Complement fixation*—This gives a positive reaction in a considerable percentage of cases.

2. *Precipitin* and *agglutination* tests are not generally used.

D. Non-Specific Tests

Sedimentation of red blood corpuscles. The sedimentation rate is increased in tuberculosis. It is useful as a confirmation test and is not diagnostic in itself. A slow rate indicates good prognosis.

E. Other Tests

1. *Estimation of opsonic index and Arneth count of blood.*
2. *Albumin test* of sputum in pulmonary tuberculosis.

BACILLUS LEPRÆ (MYCOBACTERIUM LEPRÆ)

(Hansen 1874)

Source.—Nodules or nerve sheath, in cases of nodular or anæsthetic form of leprosy, respectively; also from the nasal secretion in leprosy. They are present in large numbers, in bundles, in the granulation tissue inside the so-called *lepra cells.* They are also found in the internal organs in advanced cases.

Morphology.—Slender rods, 2—5 μ long and 0.5μ broad, usually remain in numbers arranged in masses or bundles, like cigars, and, very frequently, intracellular.

The organisms show beaded appearance, like the tubercle bacilli; and occasionally occur in branches, especially from cultures. Lepra bacillus is non-motile, and without any spore, flagella or capsule.

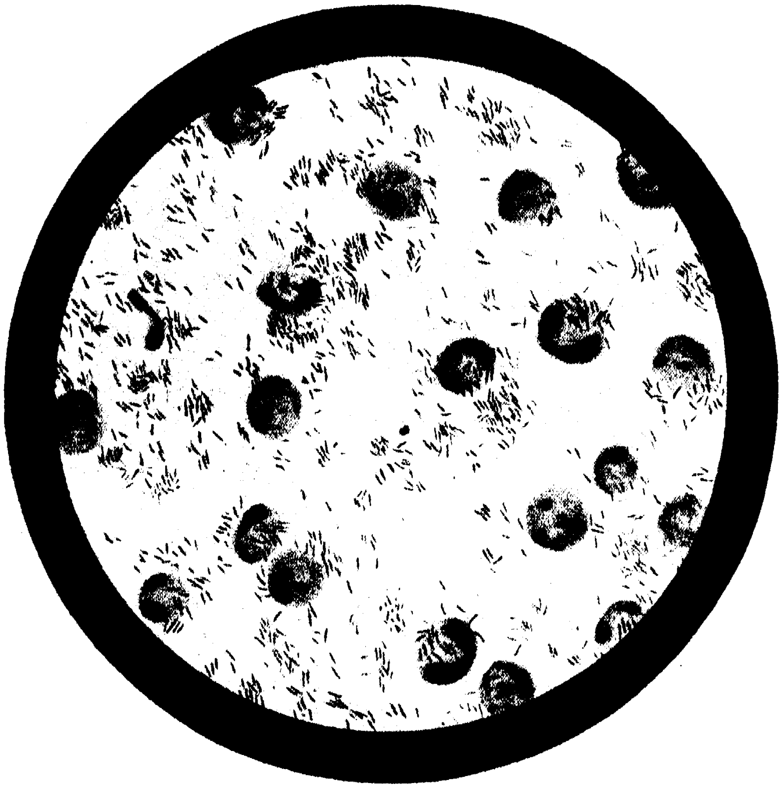


Fig. 45. Lepra bacillus from nasal smear. (Dr. Jogesh Ch. Mukherjee).

Biology.—The organism is ærobic, and grows at blood heat.

Staining Reaction.—It stains readily with aniline dyes (*cf.* tubercle bacillus). It is Gram-positive, and, when stained by Ziehl-Neelsen method, like tubercle bacillus, it retains the colour of fuchsin after decolourising with acid (watery or alcoholic solution), *i.e.*, it is an acid-fast organism. Unlike tubercle bacillus, it cannot resist strong acid. A strength of 20% $H_2 SO_4$ will decolourise B. lepræ.

The acid-fast property is said to be due to the presence of a fatty covering of the organism called ***nastin*** (a neutral fat), which is the glyceride of a fatty acid of high molecular weight.

Baumgarten claims that treatment with dilute fuchsin solution (12-15 minutes), decolourised by alcoholic HNO_3 (1:10) and counter-staining with methylene blue, stains the leprosy bacillus but not the tubercle bacillus.

Cultural Characters.—Lepra bacilli offer great resistance to growth in artificial culture medium. In fact, it is doubtful whether it has been cultivated artificially at all. Various attempts have been made for its culture but with little success. Some claim to have cultivated the organism, but, in a large number of instances, it grows as a streptothrix, or loses its acid-fast property.

Of the culture media used, the following are worth mentioning:—

(1) Rost's medium (broth, milk, and fluid from distillation of rotten fish).

(2) Kedrowski's medium.

Clegg is more successful by using alkaline meat-infusion-agar and inoculating it with amœba and symbiotic bacteria from dysentery stools. After the growth of these organisms, the culture was inoculated with the lepra bacillus.

It is found that various workers throughout the world have been successful in cultivating from leprosy four different types of organisms, namely, (*a*) acid-fast organisms forming long threads and branches, (*b*) acid-fast chromogenic bacilli forming a yellow pigment on media, (*c*) non-chromogenic acid-fast bacilli, and (*d*) acid-fast diphtheroids.

Biological Products.- On account of the difficulty to cultivate it artificially, no experiment can be made as regards the biological products of the organism.

Agglutination Reaction.—Not generally used.

Inoculation Reactions—There is a great want of any susceptible laboratory animal for successful inoculation with Lepra bacillus. The inoculation experiments invariably fail with ordinary laboratory animals; monkeys, and even human beings, have been tried but with negative or slightly positive results, which, again, are open to objections.

Pathogenicity.—It causes the disease called leprosy. The lesions are characterised by the presence of nodules in some cases, and anæsthesia with or without loss of pigment in others, thus forming the following varieties:—

(1) Nodular or tubercular form.
(2) Nervous or anæsthetic or maculo-cutaneous form.
(3) Mixed form.

Mice and rats occasionally show leprous lesions. Lepers often react to old tuberculin and give a positive Wassermann reaction.

SMEGMA BACILLUS

(Alvarez and Tavel 1885)

Source.—Found normally between the prepuce and the glans penis, between the labia or scrotum and the thighs. It is of great importance from the fact that it may be present in urine, and be mistaken for tubercle bacillus.

Morphology.—Slender rod mesuring $2\text{-}5\,\mu \times 0.5\,\mu$, nonmotile: without flagella, spore or capsule.

Staining Characters.—Does not stain readily with aniline dyes. It is Gram-positive and slightly acid-fast.

Cultural Reaction.—ORDINARY MEDIA not suitable for primary culture. BLOOD SERUM OR NASGAR MEDIA—thin, delicate, cream coloured, ropy colonies. POTATO—small greyish colonies.

Inoculation Reactions.—Non-pathogenic to animals.

Pathogenicity.—Non-pathogenic to man.

BUTTER BACILLUS, GRASS BACILLUS, ETC.

(Rabinovitsch 1897, Mœller 1898)

Source.—Butter, milk, grass, dung, etc.

Morphology, etc.—They are all slender rods, Gram-positive, acid-fast organisms. They grow readily in ordinary culture media.

Pathogenicity.—Nil.

Differentiation of the Acid-Fast Organisms

	B. Tubercl.	B. Lepr.	B. Smegma.	Others.
Morphology	Arranged singly or in pairs.	Arranged in bundles.	Arranged singly.	Arranged singly.
Staining with anilin dyes.	Difficult to stain	Readily stained.	Difficult to stain.	Readily stained.
Acid-fast character.	Can resist 20 H_2SO_4	Less acid-fast		Least acid fast.
Growth on ordinary media.	Scanty.	No growth.		Abundant growth.
Growth at room temperature.	Nil.	Nil.		Abundant.
Pathogenicity to guinea pig.	Pathogenic.	Non-pathogenic to guinea-pig if given subcutaneously; intravenously may cause suppuration.		

SPORE-FORMING ORGANISMS

BACILLUS ANTHRACIS

(Observed by Pollender in 1849, named by Davaine in 1863)

Source.—In man—(1) skin lesion in malignant pustule, (2) blood in case of septicæmia, (3) sputum in case of wool-sorter's disease, (4) spleen and other organs—post-mortem. In sheep and other animals—spleen, blood, nasal secretion, etc.

Morphology.—The bacillus anthracis is a thick rod-shaped organism, with square cut ends. It is arranged in a chain consisting of several segments. Each segment represents one organism. In tissue, the chain is very small, consisting of only 4 or 5 segments; in blood, 5 or 6 segments; but, in culture, the segments are very numerous, often amounting to several hundreds. It is not uncommon to find one colony being composed of a single chain. Various involution forms are found, especially in old culture, the shape becoming irregular and swollen with, occasionally, a granular protoplasm.

Size.—Bacillus anthracis is perhaps the biggest bacillus of importance in human pathology. Ordinarily, it is about 4-10 μ in length and about 1 μ in breadth, but it may be as long as 20 μ, and very long filamentous forms are not rare in culture.

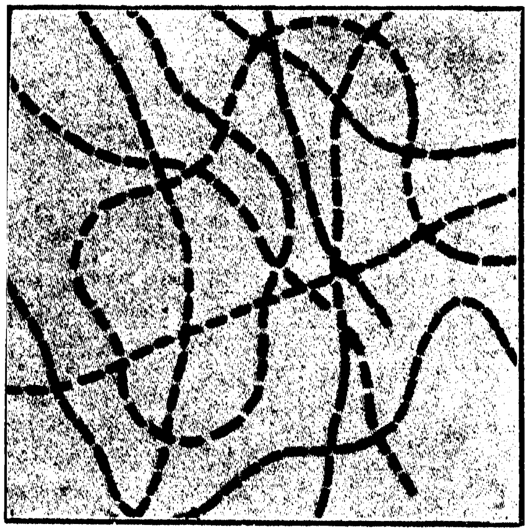


Fig. 46. B. Anthracis, smear from broth culture.

Morphology.—The organism is non-motile, and there is no flagellum. Ordinarily, no capsule is observed. It is frequently observed in culture, as well as in the tissue. In culture, the capsules often blend to form one mass when the organisms are lying close together. The bacillus anthracis is one of the spore-forming organisms.

Certain strains of B. anthracis are asporogeneous. It is believed that there are two strains of B. anthracis often present together in laboratory cultures, one spore-forming and the other non-sporulating. The spore-forming strains can be converted into asporogeneous ones by cultivating them on glycerine agar.

Spores.—Like other spore-forming organisms, bacillus anthracis possesses a single spore, and that usually in the middle, which is called "the central spore." The spore is oval, measuring about $1\text{-}5\mu \times 1\ \mu$ the long axis corresponding to the long axis of the organism.

The spores appear only when the nutritional element is deficient, or when the organism is placed in unfavourable circumstances, especially when there is free access of air. Thus, in the living animal tissue and in young culture, spores are always absent. Spores appear when the animal is dead or in old culture, provided there is a sufficient amount of air in contact with the organisms.

The spores are extremely resistant to external agencies. Prolonged boiling and very strong antiseptics are required to kill the spores, whereas the organisms can be killed easily at 55°C, or by weak antiseptics.

Dried on silk threads, they survive for 10-17 years. In old gelatin culture, they have been found alive after 18½ years. Antiformin (5-10%) rapidly dissolves both the rods and the spores. 4% formalin kills the spores in one hour. Ultra-violet rays cause their mutation.

The following shows the action of agents on the organism and the spores. They are killed by the following:—

	Bacillus anthracis.	Spores of B. anthracis.
Corrosive sublimate	1 in 10,000 in 5 minutes	1 in 500 for at least an hour.
Carbolic acid	1% in 5 minutes	5% for 24 hours.
Dry heat	100°C for 15 minutes	140°C for 3 hours.
Boiling	Few minutes	Half an hour.
Drying	Few minutes	For years.
Gastric juice	Destroyed	Not killed.

The resistance is increased with the age of the spore.

Biology.—It is an ærobe and facultative anærobe. It grows well at 37.5°C, extremes of temperature being 15°C and 45°C.

Staining Reactions.—The organism stains well with the ordinary aniline dyes, and is Gram-positive.

Spore Staining.—This is done in the following way (*Moller's method*). Fix films, treat with chloroform for 1 to 2 minutes. Wash, treat with 5% chromic acid for 5 minutes. Wash and stain as with the Ziehl-Neelsen method, as used for acid-fast organisms. Only substitute 1 or 2% sulphuric acid for decolourisation. The spores are stained red, while the bacillus is stained blue.

Cultural Reactions and Biological Products.—Bacillus anthracis grows well in ordinary media. AGAR—abundant cream-coloured growth with characteristic margin which is wavy and shows like the margin of cotton wool, hence called the " wooly margin." BROTH—becomes turbid, and gives indol reaction. GELATIN—growth similar to that of agar. Liquefaction takes place slowly in 2 or 3 days. In gelatin stab, it appears as an inverted fir tree; on BLOOD SERUM, a similar growth appears with gradual liquefaction of the medium. POTATO—similar growth. This is the best medium for the development of spores.

Inoculation Reaction.—Nearly all laboratory and domestic animals are susceptible, except the adult white rats, dogs and cats; virulence is attenuated by passage through non-susceptible animals, prolonged cultivation in artificial media, allowing it to grow at abnormal temperature (42-45°C), or by addition of a weak antiseptic to the culture media. (*See* also, production of artificial immunity.) When inoculated into a laboratory animal, it dies of septicæmia within 24-48 hours according to the virulence of the organisms.

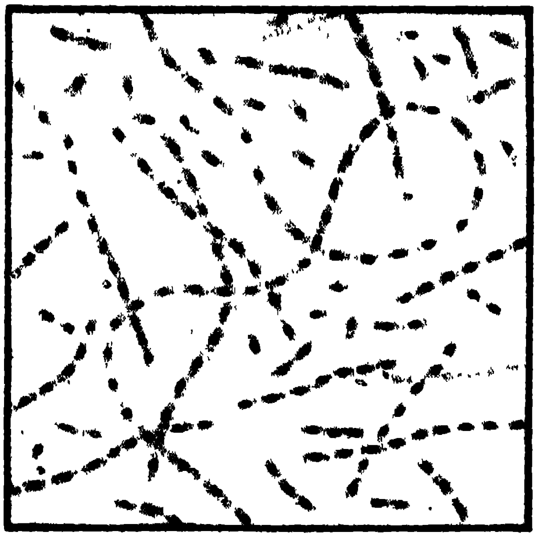


Fig. 47. B. Anthracis showing spores.

Antagonism.—B. pyocyaneus is an antagonist to B. anthracis as it is dissolved by a soluble substance (*pyocyanese*) produced by the B. pyocyaneus. Streptococcus is also antagonistic to B. anthracis.

Post-mortem.—The most prominent feature is the presence of great enlargement of spleen, which is intensely congested and contains large numbers of the bacillus.

The organisms are found in blood, spleen, muscles, kidneys, liver, lungs and other organs. Hæmorrhages in the internal organs or serous membranes are common.

Pathogenicity to Man.—Anthrax is very infectious to lower animals; man is accidentally infected. Thus, those persons who mostly come in contact with lower animals, especially sheep, (*e.g.*, shepherds, butchers, veterinary surgeons, woolsorters, tanners, etc.), are liable to get the infection.

Malignant Pustule or Charbon.—It is a cutaneous affection caused by the B. anthracis. Persons who deal with hides are mostly liable. Shaving brush is also a source of danger.

Woolsorter's Disease.—Pulmonary affection and subsequent septicæmia caused by B. anthracis. Persons who deal with sheep's wool are mostly liable to be infected.

Immunity.—Active immunity can be produced in animals by attenuated culture (allowing them to grow at 42-43°C in broth, thus producing asporogeneous strains of diminished virulence) as detailed by Pasteur.

BACILLUS MYCOIDES

Source.—Upper layers of soil and sewage.

Morphology.—Thick rods, 3-5 μ in length; resemble B. anthracis in size and shape. They are motile, forming spore, with no capsule.

Biology.—It is an ærobe, and grows at blood heat.

Staining Reaction.—Stains well with aniline dyes, and is Gram-positive.

Cultural Reaction and Biological Products.—AGAR—cream-coloured mould-like growth. BROTH—turbid, forms a pellicle on the surface. MILK—alkaline with coagulation.

Animal Inoculation.—It is non-virulent to laboratory animals.

BACILLUS SUBTILIS

Source.—Hay, soil, dust, etc.

Morphology.—2-4 μ, slender rods and filaments, motile, spore-forming, no capsule.

Biology.—Ærobe, growing at 37°C.

Staining Reaction.—Stains with ordinary dyes, and is Gram-positive.

Cultural Reaction.—AGAR—moist, cream-coloured wrinkled growth. BROTH— turbid. GELATIN—same as agar with liquefaction. MILK—alkaline with coagulation.

BACILLUS TETANI (CLOSTRIDIUM TETANI)

(Nicolaïer 1885)

Source.—Garden earth, excreta of horses, cattle. In man—the seat of inoculation.

Morphology.—The organism is slender straight rod, or in filaments with rounded ends. It is motile, but the motility suspends rapidly by the action of light during observation under the microscope. It contains numerous flagella attached over the whole surface of the organism excepting the ends. Some varieties are non-motile and possess no flagella. It forms spores which are spherical, and are placed terminally, *i.e.*, at the extremity of the rod, assuming a drum-stick appearance. It possesses no capsule. The spores are highly resistant and can resist boiling for 5 minutes.

Biology.—It is a strict anærobe, and will not grow in presence of a minute amount of free oxygen. It grows best at 37.5°C, minimum temperature being 14°C.

Staining Reactions.—It stains well with aniline dyes, and is Gram-positive.

Cultural Reactions.—Must grow anærobically. GLUCOSE AGAR stab—grows abundantly, cream-coloured, margin feathery (the so-called "inverted pine-tree growth") with formation of a little gas. There is no growth on the surface. BROTH—turbid. SERUM—not liquefied. MILK—no coagulation. GELATIN—liquefied slowly. ROBERTSON'S BULLOCK'S HEART medium—grows well.

Isolation of B. tetani.—This is based on the principle that the organisms form spores, and that the spores are resistant to heat. Exposure to a temperature of 80°C. for one hour will not kill the spores, but will be quite sufficient to kill all organisms including the B. tetani themselves. The suspected material is inoculated into a glucose-agar tube and both the B. tetani and other contaminating organisms are allowed to grow for 48 hours in anærobic condition. By this time a large number of spores will have developed. The culture tube is then placed in a water bath at a temperature of 80°C. for one hour, which will kill all organisms except the spores. The material from this culture medium is then cultivated anærobically.

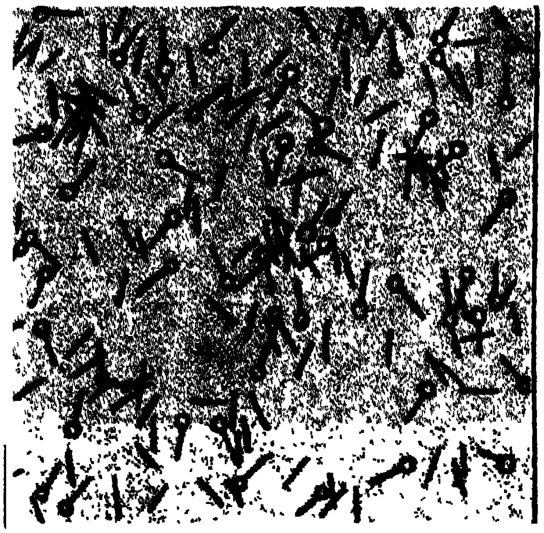


Fig. 48. B. tetani showing spores.

It is very difficult to isolate B. tetani when other spore-forming organisms are present in the material.

Agglutination Reactions.—Recently, eight types of tetanus bacilli have been established by immunity reactions. Sera made from Types I, III and V are specific. Types VI, VII and VIII are rare.

Inoculation Reactions.—All laboratory animals, except the fowls, are susceptible. The tetanus bacillus is always found in the seat of inoculation, or up to the nearest lymphatic gland, and never beyond that. The organisms produce toxin, which is absorbed and leads to the symptoms. The toxin is absorbed through the motor end apparatus and traverses centripetally by the axis-cylinder of the motor nerves to the spinal ganglia of the affected side, then of the other side, and ultimately to the brain. If the toxin is introduced into the blood, it enters the central nervous system through the motor nerves.

Experiment.	Result.	Inference.
Pure culture of B. tetani injected into animal.	No tetanus	Organisms destroyed.
Pure culture of B. tetani mixed with culture of staphylococcus.	Tetanus	Staphylococcus absorbs oxygen from tissue to make it anærobic for B. tetani to grow and produce toxin.
Pure culture of B. tetani with lactic acid.	Tetanus	Surrounding tissue is damaged by lactic acid rendering it anærobic.
Spores from culture	No tetanus	Spores can remain for years in the tissue.
Spores with quinine	Tetanus	Quinine producing necrosis of tissues makes latter anærobic and hence tetanus.
Garden earth	Tetanus	The garden earth contains both spores of B. tetani and other septic organisms. The latter produce tissue necrosis.

Pathogenicity.—The bacillus tetani causes the disease known as tetanus. The affection is caused by the absorption of the toxin produced by the organism, the organism never occurring in the circulating blood or in the nervous system. In man, the spasm first affects the extensor muscles of the trunk, whereas, in other animals, the muscles in the neighourhood of the site of infection are affected first.

Seat of Infection.—(1) Any wound or ulcer on the surface of the skin. (2) Dysenteric and other ulcers of intestines. (3) Minute, even microscopical abrasions may serve as the portal for the entry of the organisms. (4) Umbilicus of a new-born child.

Source of Infection.—(1) Earth, dung, or any substance contaminated with them. (2) Catgut ligatures, in infection through operation wounds.

Distribution of the Organisms.—The organisms are present only in the local lesion. They may be present very rarely (if at all) in other parts of the body.

The Toxin.—Tetanus bacillus produces a very powerful extracellular toxin when grown in broth in an anærobic condition. That it is extracellular, is known by the fact that, when the culture media containing the growths of the organism is filtered though a Pasteur-Chamberland filter, so as to free it from all organisms, a very minute dose of the filtrate is very highly toxic to a guinea-pig, leading to a fatal result. It is about twenty times as toxic as dried cobra venom. The tetanus toxin is very labile and is destroyed easily by physical and chemical agencies.

The Nature of the Toxin.—The toxin has got a special affinity for combining directly with nervous tissue. Hence the nerve cells of the spinal cord are affected, resulting in spasms and other motor disturbances. It has no action on the sensory nerve endings. The toxin is composed of two substances, (*a*) a spasm-producing substance called *tetanospasmin,* and (*b*) a hæmolysin called *tetanolysin.*

The path of absorption of toxin.—Experiments:—

1. The toxin is introduced into the muscles of the hind limb of a rabbit, sciatic nerve of the same side being cut near the spinal cord. The sciatic nerve is excised after some hours and introduced into a mouse which subsequently dies of tetanus.

2. The same experiment is repeated with the sciatic nerve being cut near the muscles. The proximal part of the nerve is harmless, although it was surrounded by lymph which contained the toxin.

This shows that the toxin is absorbed through the end-plates in the muscles, and not by the lymph surrounding the nerve.

That it is not absorbed through the sensory nerve ending, is shown by the fact that introduction of a lethal dose into a pure sensory nerve, *e.g.*, the infra-orbital, does not produce any symptom.

Meyer and Ransom believe that, whatever may be the mode of introduction—subcutaneous, intramuscular, intravenous or otherwise, it is taken to the nervous system, being absorbed only through the end-plates in the muscles. From this, it is taken up by the motor nerve sheath to the spinal cord. They are of opinion that sometimes affection of the sensory nerves takes place, the condition being termed "tetanus dolorosus" or the "painful tetanus." In this condition there is no motor spasm, but there are great hyperæsthesia and hyperalgesia.

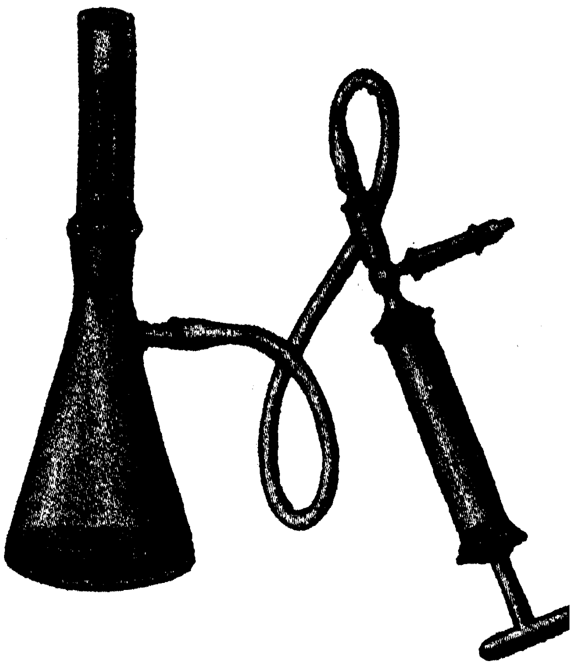


Fig. 49. Pasteur-Chamberland filter.

The Causation of Symptoms occurring in Tetanus.—The disappearance of contractions by curare and deep anæsthesia on experimental animals shows that the effect of the tetanus toxin is not on the muscles. The contractions are stopped by section of the central ends of the nerves and also stop progressively from above downwards when the cord is destroyed in this direction, while section of the cord alone does not stop them. These show that the toxin acts on the central ends of the nerves. The lesion is mainly a disturbance in the reflex arc joining the sensory and motor apparatus in the spinal cord.

Post-mortem Changes.—Muscles show hæmorrhages, which are probably due mechanically to spasms. The central nervous system shows general congestion, especially of the grey matter, which may also extend to the white matter; minute hæmorrhages are also common. Anterior cornual cells show degenerative changes. There is no definite change in any other tissue or organ of the body.

Antitoxin.—On account of the extracellular nature of the toxin, it can be injected into a horse in increasing doses with production of a very high degree of immunity in the horse. The serum of the immunised animal contains a very powerful antitoxin. The antitoxin has got a great prophylactic value, but possesses little curative property.

Still, antitoxin treatment must be continued, as it may help a good deal, especially in a subacute case.

Behring and Kitasato first produced artificial immunity of animals and showed that the immunity was due to substances in the circulating blood.

The Anærobic Bacilli of Infected Wounds

These organisms cause the so called "gas gangrene" which was specially prevalent during the Great War. They generally remain in the animal excreta, and when they contaminate the wound, they produce rapidly spreading inflammatory œdema, necrosis and gas. They are all anærobic and may be divided into two groups:—

(1) The saccharolytic group, *e.g.*, B. welchii, Vibrion septique,

(2) The proteolytic group, *e.g.*, B. sporogenes, B. tetani, etc.

In the Bullock's heart medium, the saccharolytic group produces abundance of gas (CO_2 and H_2) and acid with no digestion of the medium, whereas the proteolytic group digests the meat with formation of foul-smelling sulphur compounds which blacken the meat. They have very little action on carbohydrates.

Proteolysis implies the property of these organisms to attack higher proteins, *e.g.*, serum, gelatin, egg white, fibrin, casein, etc. Of all the anærobic organisms, B. welchii, on account of its greater frequency of occurrence, has received more attention. These organisms occur not only in the military but in civil practice as well. The more extensive the wound or injury, the greater the infection. The infection may be localised if the injury is less serious. Generalised fulminating and rapidly fatal infection may take place in septicæmia, severe puerperal sepsis or contamination of wound with bowel contents, appendicitis, etc.

Antitoxins have been prepared of all the organisms associated with gas gangrene. It has been found, on experimental animals as well as in practice during the War, that any adequate dose of the antitoxin injected immediately after the receipt of the wound, may prevent the development of gangrene, but administration of antitoxin after the development of the lesion invariably fails to protect the experimental animal as well as the human patient. From these it is evident that, to be effective, the antitoxin must be given very early and in big doses—50-100 c.c. of unconcentrated serum or 40-50 c.c. of concentrated antitoxin.

Frequently B. œdematis maligni and B. welchii are often present together in wounds and abdominal conditions; so the sera containing antitoxins to both these organisms may be administered with advantage both prophylactically and therapeutically, in civil as well as in military practice.

BACILLUS ÆROGENES CAPSULATUS

(Welch and Nuttal 1892)

(*Also known as Gas Bacillus, B. welchii and B. perfringens.*)

Source.—Soil, dust, intestinal contents, etc.

Morphology.—Rod shaped, 4-5 μ in length occurs singly, in chains or clumps. It is non-motile, and possesses no flagellum. It occasionally forms spores, specially in serum and cooked meat media. It forms no spore in ordinary media, and rarely in tissue. A capsule is present.

Biology.—It is a strict anærobe, and grows well at 37.5°C.

Staining Reaction.—Stains well with aniline dyes, and is Gram-positive.

Cultural Reaction and Biological Products.—Grows readily in anærobic condition, and on media containing carbohydrate, *e.g.*, glucose agar. AGAR—greyish-white opaque colonies. SERUM AGAR—grows in abundance. BROTH—turbid. MILK—coagulated and produces an odour of butyric acid. POTATO—invisible growth GELATIN is liquefied. COOKED MEAT MEDIUM.—Colour is changed to pink with formation of large quantity of gas. No digestion of the meat occurs and so no blackening of medium nor any putrid odour in the culture medium.

Sugars.—Majority of sugars are fermented with production of gas and acid.

Simonds first recognised that there are four groups or strains of B. welchii grouped according to their action on carbohydrates:—

Group I—ferments inulin and glycerine.
Group II - ferments glycerine and not inulin.
Group III - ferments inulin and not glycerine.
Group IV—does not ferment glycerine nor inulin.

Proteolysis.—B. welchii is essentially a saccharolytic organism; the proteolytic properties are very limited.

Agglutination Reaction.—Positive in some cases.

Inoculation Reaction.—Laboratory animals are susceptible. Fatal to guinea-pig in 48 hours. Pigeons are the most susceptible laboratory animals. *Subcutaneously.*—Leads to gangrene with extensive production of gas and exudation leading to excessive disintegration of tissue with partial digestion. *Intravenously.*—Rapidly fatal. If the dead body is incubated, extensive gas production occurs in the internal organs—especially the liver. (*Welch-Nuttall test.*)

Pathogenicity.—In human beings this is the commonest cause of what is known as gas gangrene, most common in war wounds. Rapid and extensive gangrene takes place with production of gas, chiefly from the carbohydrates of the muscles. In terminal stages, the organisms enter the blood, with formation of numerous metastatic gangrenous foci. It often remains normally in the intestines and may cause infections of the uterus (septic abortion), alimentary tract or the urinary organs.

BACILLUS SPOROGENES

(Metchnikoff 1908)

Almost identical with B. ærogenes capsulatus in morphology, biology, staining and cultural reactions with the following differences:—

	B. æorgen. cap.	B. sporogenes.
Motility	Nil	Present.
Flagella	Nil	Present.
Capsule	Present	Absent

BACILLUS (CLOSTRIDIUM) ŒDEMATIS MALIGNI

Syn.—*Vibrion septique* (*Pasteur*), *Bacillus of Malignant Œdema* (*Koch*).

Source.—Gas gangrene following septic wounds.

Morphology.—The bacillus œdematis maligni is a slender rod, arranged singly, or in filaments. It is motile, and possesses numerous flagella. It forms subterminal or central spores and possesses no capsule.

Biology.—Grows anærobically at 30°C.

Staining Reactions.—Stains well with aniline dyes, and is Gram-positive.

Cultural Reaction and Biological Products.—AGAR—thick greyish-white growth with irregular margin; gas is produced in abundance BROTH—turbid, with production of gas. The organisms sink down, leaving the upper part of the fluid clear. MEAT MEDIUM—gas formation takes place with change of colour to pink but no blackening.

Sugar Reaction.—It is a saccharolytic, non-proteolytic organism. It ferments actively glucose, lactose and maltose. Proteolytic properties are very limited.

Inoculation Reaction.—Ordinary laboratory animals are susceptible. Inoculated subcutaneously, it leads to a fatal result. The seat of inoculation is œdematous, hæmorrhagic and inflated with gas, and the spleen is slightly enlarged with accumulation of a large number of the organisms under the capsule. Very few organisms are present in the blood, or other organs.

Toxin.—V. septique secretes a filtrable toxin, producing necrosis, paralysis and convulsion.

Pathogenicity.—Unlike B. welchii, B. œdematis maligni shows little or no variation in regard to the pathogenicity, even when grown upon artificial media for years together. Subcutaneous inoculation in guinea-pig kills the animal in 12-24 hours, with extensive œdema and formation of gas in the tissues. Spontaneous recovery is unusual. Extensive hæmorrhagic œdema, deep red colour of the muscles and gas are noticed post mortem. The muscles are not soft and friable, nor any putrid odour is present. In man a fulminating gangrene is produced, with extensive lesion in the site of injury and various organs, especially the liver. The cause of death is the toxin itself elaborated by the bacteria. It produces gas gangrene with putrefaction, œdematous condition and subcutaneous emphysema of the part.

BACILLUS (CLOSTRIDIUM) CHAUVŒI

This is an organisim belonging to those associated with gas gangrene and causes a disease of the cattle, commonly known as *blackleg.*

BACILLUS BOTULINUS

Source.—Food, especially meat, causing poisoning, the commonest being fish, sausage, ham and bottled fruits.

Morphology, Biology and Staining Reaction.—Large bacillus occurring either singly or in pairs. It is anærobic and Gram-positive. optimum temperature 20°-30°C. Terminal ovoid spores are present.

Cultural Reaction.—Agar and Broth—same as B. œdematis maligni. Gelatin is liquefied with production of gas.

Toxin.—In broth containing 2% glucose, it produces a potent exotoxin and other toxic substances. An efficient polyvalent antibotulinus serum has been prepared.

Pathogenicity.—Meat poisoning. It produces a potent exotoxin and causes a form of food-poisoning in man called *botulism.* This takes place not from the infection with these organisms but from the ingestion of the pre-formed toxin of the same. *It is, thus, an intoxication.* Experimental evidence shows that the organisms appear to have no capacity to infect either locally or generally.

The main symptoms are disturbance of vision, paralysis of ocular nerves, dysphagia, dryness of throat and death from cardiac or respiratory failure. Acute gastro-intestinal disturbances are usually absent. As a rule, constipation is present.

BACILLUS OF SYMPTOMATIC ANTHRAX

Source.—Sheep or cattle suffering from blackleg.

The organism is motile, and resembles B. botulinus.

BACILLUS BUTYRICUS

Source.—Milk. Organism similar to B. botulinus.

Pathogenicity.—Nil.

BACILLUS DIPHTHERIÆ (CORYNEBACTERIUM DIPHTHERIÆ)

Klebs *observed and described it in* 1883, **Lœffler** *obtained it in pure culture in 1884.)*

Source.—Diphtheritic membranes and secretions of nose and throat, conjunctiva, external genitalia, specially vulva. It may also occur in gunshot injuries.

Morphology.—The bacillus diphtheria is a slender rod, with rounded ends, measuring 2-5 μ in length. The wide variations in length together with irregular development of the swellings give rise to polymorphism. Besides the form descrived above, in normal culture, coccal, streptococcal, sheathed, barred and branching forms may be found. The organisms are often arranged in parallels, being placed side by side. In the membrane they are found abundantly in the more superficial layers, usually in clumps. The deeper layers contain few or no bacteria.

Involution forms are common, according to the culture media used and the age of the growth. The forms vary, from very short forms to very long ones, occasionally clubbed. In stained specimens, it shows beaded or segmented appearance.

Biology.—It is an ærobe and a facultative anærobe. Optimum temperature, 37.5°C.

Staining Reactions —Stains well with aniline dyes, and also with Gram's method. It seems to have a special affinity for alkaline methylene blue (Lœffler's). By this stain the granules become prominent and often appear as streptococci. The granules are better demonstrated by the Neisser's stain. Neisser's stain contains acid methylene blue.

Special Stain.—NEISSER'S STAIN.—Stained by this method, the metachromatic granules are stained deep blue, whereas the body of the organism is stained light brown. Sometimes the granules are placed only at the ends, which are deeply stained—*the so-called polar staining.* The Neisser's stain shows the granules better when the organisms are collected directly from the membrane or from culture grown on egg medium. Agar culture shows poor results. Branching forms and even coccal forms are found in cultures.

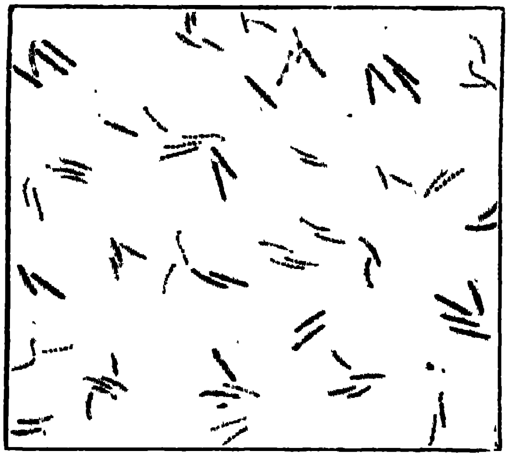


Fig. 50. B. diphtheria. Smear from culture

The presence of the polar staining and of the metachromatic granules by Neisser's stain, differentiates diphtheria from pseudo-diphtheria bacillus.

From the appearance of the granules in the organisms three main groups can be made out: (*a*) Granular forms showing distinct spherical or ovoid granules; (*b*) Barred forms showing alternating dark and pale areas; (*c*) Solid forms.

Cultural Reaction.—AGAR.—Moist, cream coloured, opaque growth. The centre of the colony appears yellowish white and the edges are white. BROTH Turbidity. It settles at the bottom with formation of a powdery layer on the side of the tube.

SPECIAL CULTURE MEDIA.—LŒFFLER'S BLOOD SERUM MEDIUM.—In this medium, the growth is very rapid, often forming visible isolated colonies within 6-8 hours, long before the appearance of the colonies of other organisms present in the throat. The colonies are of opaque white or whitish-grey colour. These colonies are smaller than those of staphylococci but larger than those of streptococci. With a magnifying lens, they appear distinctly granular. EGG MEDIUM.—Same as blood serum medium. TELURITE EGG MEDIUM (Sodium telurite, 0.1 grm.; Distilled water, 10 c.c.: add 1 c.c. of above to 25 c.c. of ordinary egg medium.) produces characteristic black colonies. GELATIN.—No liquefaction. MILK turns acid, but no coagulation.

Toxin.—A powerful extracellular toxin is obtainable by filtering the broth culture. Although the filtrate is sterile, still the effect is the same as that of the living culture. The products of the organisms consist of three substances:—

(1) PURE TOXIN.—Causes the acute manifestions. It was first obtained by Roux and Yersin. Injection of pure toxin produces exactly the same manifestion as with living organisms. This shows that the living organisms act through this toxin. The toxin is thermolabile and becomes inert when heated to a temperature of 58-60°C., for half an hour. It can be dried without harm, but when dried it becomes more resistant to heat (even 100°C., for 20 minutes).

The diphtheria toxin can be precipitated, concentrated and purified by saturation with ammonium sulphate, alum and other salts, also by addition of one to three per cent. acetic acid and then collection of the precipitate and resolution in caustic soda solution; all operations being carried out at the freezing point.

(2) TOXOID—Derived from the former due to deterioration with age, and is not itself toxic, but takes part in the production of antitoxin when injected.

(3) TOXONES.—These produce late manifestations, such as the nerve lesion (paralysis) in man or experimental animals.

All the three substances combine with antitoxins.

Antitoxin.—Diphtheria antitoxin has been discovered by von Behring and is obtained from horse after repeated injections of standard diphtheria toxin.

Agglutination Reaction.—The organisms are agglutinated by high titre serum, as well as by the serum of the patient, but it is not of importance in diagnosis or in the differentiation from the pseudo-diphtheria bacilli.

Inoculation Reactions.—All laboratory animals, except mice and rats, are susceptible. Inoculated into the throat, conjunctiva and vagina of rabbits and guinea-pigs, it leads to the formation of a pseudo-membrane. Occasionally, it is found that the healthy mucous membrane is not susceptible, unless it is injured. The number of the organisms rapidly decrease after experimental introduction into the animal. Thus, death takes place only after a considerable time in the second animal inoculated with the membrane or œdema fluid of the first one, while the third one inoculated from the second usually survives.

Toxin derived from the broth culture of the bacillus, is fatal to guinea pig. The inoculation of bacillus diphtheria into healthy cats produces similar disease. Small amount of virulent culture injected into a guinea-pig will produce symptoms of illness in 12-24 hours with loss of appetite and unwillingness to move. The site of injection is swollen, infiltrated and sensitive to pressure. Highly virulent culture will cause death in 2-4 days from dyspnœa. At autopsy the site shows greyish necrotic masses containing diphtheria bacilli. The neighbouring lymphatic glands are swollen and hæmorrhagic. The peritoneal cavity shows a serous hæmorrhagic exudate with congested blood vessels. The internal organs, specially the adrenals, are enlarged, congested and often hæmorrhagic. Ulcers may occur in the stomach and intestines. Congestion of adrenals and, in many cases, hæmorrhage in the adrenals are the most conspicuous lesion of experimental diphtheria.

Pathogenicity.—Bacillus diphtheria usually affects children, with the formation of a pseudo-membrane of the throat, which may extend up to the nose, larynx, trachea, conjunctiva, etc. The vaginal mucous membrane is also affected.

The organisms are present in clumps in the fibrin forming the superficial part of the false membrane. They are much less in the deeper layers. They are also found in pneumonic areas, spleen, liver and other organs after death. Streptococci, staphylococci, pneumococci and other organisms may co-exist with the diphtheria bacilli. In experimental animals Cl. diphtheria remains localised but kills it by the absorption of toxin.

Veld Sore. A form of ulcer found in the tropical countries. It is said to be diphtheritic in origin.

Laboratory Diagnosis of Diphtheria

The greater part of responsibility in the diagnosis of diphtheria rests on the clinician alone and the responsibility for diagnosis cannot be placed on the bacteriologist. The bacteriological findings may be of utmost value to the clinician, but it must be taken as only one of the factors in the diagnosis of the case. The administration of antitoxin should never be delayed in any case in which the disease is suspected.

Methods of diagnosis:—

1. Direct examination of the smear.

2. Cultural methods. For this purpose selective media are desirable. Media such as, tellurite egg, Douglas's medium containing trypsinised serum agar or trypsinised serum tellurite copper sulphate agar are most suitable. The metachromatic granules are most well seen in smears from cultures, as stained by Neisser's stain. These are of greatest help in distinguished Cl. diphtheria from Cl. hofmanni.

3. Fermentation test:—

Three sugars are generally employed for the purpose. These are glucose, galactose and saccharose. One per cent. of these sugars in peptone water with litmus or bleached acid fuchsin as indicator is used. The majority of diphtheria strains are quite constant in their reactions; they produce acid in glucose and galactose and never in saccharose. The Hofmann's bacillus is also quite constant in producing no acid in these three sugars.

4. *Animal inoculation*:—(*a*) it produces hæmorrhages in the adrenals. (*b*) Intracutaneous tests have been used for testing of virulence of the bacillus and for the presence of minute amount of antitoxin in the serum. The technique of *Virulence* test is as follows:—

The organism is first isolated in pure culture. Two colonies are picked up and inoculated in Lœffler's serum agar slopes. After 18 hours' incubation the growths are emulsified in saline and standardised to 500 million organisms per c. cm. Two guinea pigs are taken. One of them is *protected* by an intraperitoneal injection of 500 units of antitoxin. This animal serves as control. Both of these animals (the control and the unprotected one) are injected intracutaneously, after depilation, with 0.2 c.c. of the standardised suspension. About 6 hours after the injection a dose of 125 units of antitoxin is given to the unprotected animal. The lesion on the test animal (the unprotected one) produced by virulent culture consists of an erythematous patch, about 15 m.m. in diameter which progresses to superfecial necrosis within 48 to 72 hours, becoming a dry scab a day or two later.

Differential Points Between Diphtheria and Diphtheroid Bacilli.

Diphtheria	*Diphtheroid*
Weakly Gram positive	Strongly Gram positive
Facultative anærobic	Strict ærobe
Broth—grows on the surface as well as down the sides of the tube	Grows on surface of broth only

Diphtheria	*Diphtheroid*
Metachromatic granules present	Metachromatic granues absent in Hofmann bacillus which is the commonest diphtheroid.
Pathogenic to guinea pig producing hæmorrhagic adrenalitis and dermal reaction.	Not so. Virulence test negative even without antitoxin.
Sugar reactions in the majority of strains constant. They produce acid in glucose and galactose but not so in saccharose.	Hofmann bacillus does not produce any acid in these sugars. Xerosis B. Produces acid in glucose and saccharose.

Schick Test (*Schick of Vienna* 1913).

This test, which consists in injecting a minute quantity of diphtheria toxin intradermally, indicates the presence or absence of natural or artificial immunity in a person A positive test (presence of local reaction) indicates absence of antitoxin in the circulation, and a negative test indicates presence of sufficient amount of antitoxin to protect against the toxin. This test reveals the presence of about 1|60-1|40 unit of antitoxin per c.cm. of the serum of the individual.

Dilute the standardized toxin with 0·5% carbolic acid, so that 0.1 c c. contains 1|50 of minimum lethal dose for a guinea-pig weighing 250 grammes. Inject 0.1 c.c. intradermally into the flexor surface of the fore-arm. Inject the same amount of the toxin *heated to* 75°C. *for one hour* as control. A red area indicates a positive reaction which often lasts for about a week and may have brownish pigmentation.

Result— A *negative* result means that the patient is immune while a *positive* result means that the patient is susceptible. Carriers (either convalescent or contact) may carry virulent bacilli in their throats but are immune to diphtheria. They are always Schick-negative.

Importance of the Test:—

(1) It shows the amount of immunity present in a case.
(2) It shows the cases requiring antitoxin for prophylactic purposes.
(3) It recognises the true diphtheria carriers as they give negative reactions.
(4) It shows the duration and intensity of immunity following injection of antitoxic serum.

Pseudo-Diphtheria (Diphtheroids), and Diphtheria-like Organisms

These are:—

1. Hofmann's bacillus.
2. Xerosis bacillus.
3. Vincent's fusiform bacillus.
4. Acne bacillus.

HOFMANN'S BACILLUS

(*First observed by* **Lœffler 1887** *and investigated by* **Hofmann 1888**)

Source.—Throat in angina-like conditions, scarlet fever, etc.

Morphology.—Short rods 1-2μ in length. Usually arranged in pairs, non-motile, possesses no flagella, no spore, and no capsule.

Biology.—It is a strict ærobe and grows at 37°C.

Staining Reaction.—Stains well with aniline dyes as well as by Gram's method. Segmentation and polar staining absent, especially with Neisser's stain.

Cultural Reaction.—In SOLID MEDIUM, it forms cream-coloured moist colonies. Involution forms are rare. MILK—alkaline, and no coagulation.

Agglutination.—Positive, but not diagnostic.

Inoculation Reaction.—Non-pathogenic to guinea-pig.

Pathogenicity.—Pseudo-membranous condition of the throat.

XEROSIS BACILLUS

(Neisser and Kuschbert 1884)

Source.—Conjunctiva in xerosis and follicular conjunctivitis.

It closely resembles diphtheria bacillus with the following exceptions: (1) Neisser stain is negative; (2) it does not grow on agar, except in subcultures; (3) it does not produce acid in milk; (4) it is non-pathogenic to guinea-pig; (5) it requires blood serum media for growth.

VINCENT'S FUSIFORM BACILLUS

Source.—Sore throat, noma, angina etc.

(1) It is fusiform in shape with pointed ends; (2) it is longer than diphtheria bacillus, 6-12 μ in length; (3) it is somewhat bent and slightly motile; (4) it is Gram-negative; (5) it is always associated with spirochætes and grows anærobically in blood serum or ascitic fluid media. (*Also see spirochæta vincenti.*)

HÆMOPHILIC GROUP

BACILLUS INFLUENZA (HÆMOPHILUS INFLUENZA)

(Pfeiffer and Kitasato 1892)

Source.—Expectoration and nasal secretion of patients suffering from influenza, rarely from blood and cerebro-spinal fluid.

Morphology.—A minute rod, 0.5-1 µ in length, occurring singly, in chains or in groups. It is non-motile, there is no flagella. No spore, and no capsule; involution forms not uncommon.

Biology.—Strictly ærobic and grows at 37.5°C.

Staining Reaction.—Stains well with aniline dyes, and is Gram-negative.

Cultural Reaction and Biological Products.—Ordinary AGAR and BROTH.—No growth. Grows only in symbiosis with other organisms, *e. g.*, staphylococcus aureus. BLOOD AGAR (*specially with pigeon's blood*).—Minute, discrete transparent colonies. A 24 hour's growth requires a lens to be clearly seen. GELATIN—Liquefied.—It possesses a very low vitality in culture, and dies rapidly outside the body.

Inoculation Reaction.—Difficult to get any definite effect by inoculation to laboratory animals.

Pathogenicity.—Causes influenza, endocarditis, meningitis, etc.

THE ETIOLOGY OF EPIDEMIC INFLUENZA.—Apart from the part played by B. influenza in causing pneumonia during an epidemic, the exact etiological agent has called a great controversy, in which *the filter-passing virus* as a causal agent is a strong factor. This is based on the following facts:—

Experimental Evidence.—Washings from the nose, influenza sputum and lung juice have been filtered and the filtrate sprayed into the nose and injected subcutaneously or even intravenously. These experiments on man do not give any support to the *filter-passer* hypothesis, as well-conducted cases show a negative finding, and those cases showing positive results are regarded to be fallacious, as the experiments were done at the height of the 1918 epidemic, with no isolation of the subjects. Similar sets of experiments on animals, including monkeys, again, where rigid control is not so difficult as that of man, also give inconclusive results.

The 1915 *Epidemic in America.*—Detroit (U. S. A.) had 100,000 cases of influenza-like illness in a population of 500.000 in the month of December 1915. During this epidemic, 18 per cent. of the typical cases showed the presence of influenza bacillus. This epidemic was the forerunner of *the* 1918 *pandemic,* which was responsible for 6 million deaths in India alone, with a figure of more than 15 million throughout the world.

Bacteriological Findings in the Pandemic.—The pandemic was observed to appear in three waves. The first wave shows absence of B. influenza in all countries, streptococci and pneumococci being the prominent organisms. But, in the second wave, a high percentage of B. influenza present was associated with a wave of high mortality.

From these observations, two schools have originated regarding the interpretation of the findings. Some believe that the Pfeiffer's bacillus is an epiphyte, and a secondary invader multiplying in normal throats but may produce catarrh. The other school regards B. influenza as the primary cause of the pandemic influenza. The general opinion, however, is against the Pfeiffer's bacillus as the causative organism. Mixed infection with pneumococcus and streptococcus also plays a very important role.

BACILLUS PESTIS (PASTEURELLA PESTIS)

(Kitasato and Yersin 1894)

Source.—*In man.*—(*a*) Smear from bubo; (*b*) Blood; (*c*) Expectoration in cases of pneumonic plague; (*d*) Spleen and other organs—post mortem. *In rats.*—Blood and other organs. *In rat-flea.*—Alimentary tract especially stomach and proventiculus.

Morphology.—It is a short, thick, pleomorphic organism. The pleomorphism is exhibited by forms like staphylococci, streptococci, moulds or even yeasts. It measures 2-3 μ by 1-2 μ. Involution forms occur. It is non-motile, non-sporing, non-flagellated and non-capsulated. Smear from peritoneal exudate of guinea-pig often shows a viscid capsule. Gordon found flagella, but they have not yet been confirmed.

Biology.—It is an ærobe, and a facultative anærobe, and grows at 37·5°C, but grows best at 25-30°C.

Staining Reactions.—Stains well with aniline dyes, and is Gram-negative. In a stained preparation, the appearance is characteristic. The two poles are deeply stained, leaving the middle portion unstained —*the so-called bipolar staining.* The bipolar staining can often be better seen by first treating the film for ½ minute with ½% acetic acid, then treating it with absolute alcohol, evaporating it by holding it on the flame and then staining with dilute aqueous methylene blue or carbol fuchsin. In tissue, the section should be fixed by alcohol and then stained by Unna's polychrom methylene blue.

Cultural Reactions.- -Grows best on blood serum medium. BLOOD SERUM MEDIA.—Thick, opaque, smooth, moist, cream-coloured growth. AGAR.—Similar growth which is sticky and tenacious. SALT AGAR (3% NaCl).—Involution forms usually pear-shaped or spherical. GELATIN.—Thick growth with irregular margins but with no liquefaction. MILK.—Not coagulated. BROTH.—Takes time to grow, turbid after some days. The cultures become alkaline and autolysis of the organisms takes place after a few days in broth. GHEE BROTH.—Broth remains clear, flocculent growth like strings of beads appears from the under surface of the ghee (or oil) and hangs in the broth—*the so-called stalactite growth.* This appears in about a week, and only when the broth remains undisturbed.

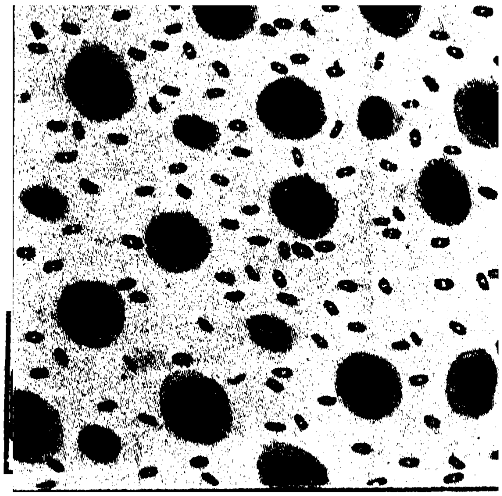


Fig. 51. B. pestis.
Smear from bubo.

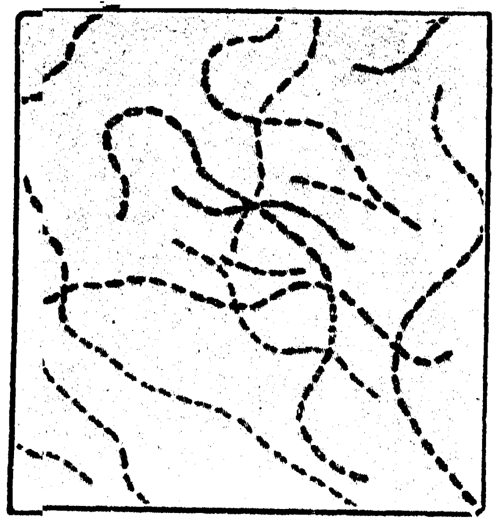


Fig. 52. B. pestis.
Smear from broth culture.

Inoculation Reaction.—Ordinary laboratory animals are all susceptible to B. pestis. GUINEA-PIG—fatal in 2-7 days. POST MORTEM—extensive hæmorrhagic œdema at the seat of inoculation. SPLEEN—greatly enlarged and congested, studded with numerous small nodules full of the bacilli surrounded by areas of necrosis. GLANDS.—Enlarged with numerous hæmorrhage.

Mode of Infection.—This has been definitely established by the Advisory Committee appointed by the Secretary of State for India in 1905:—

Some of the experimental facts are given below:—

1. Plague rat and healthy rat placed in adjacent cages—

(*a*) If fleas are present—healthy rat becomes infected;
(*b*) If fleas are absent—no infection;
proving that the infection takes place through the flea, and not through air.

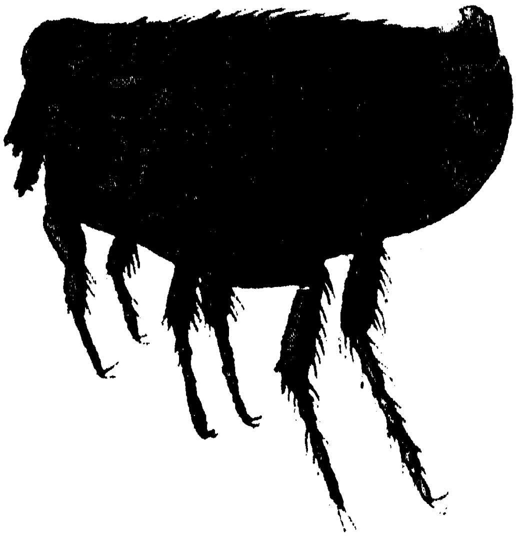


Fig. 53. Rat-flea.

2. Fleas removed from plague rats and put on healthy rats—infection takes place; proving that the fleas directly transmit the infection.

3. Plague-infected guinea-pigs and healthy guinea-pigs placed in one cage—
(*a*) If uninfected fleas are introduced—infection takes place; (*b*) If fleas are absent--no infection; proving that no infection takes place by direct and close contact.

4. Plague-infected guinea-pig with fleas on them are allowed to run about on the floor. and healthy guinea-pigs are suspended in a cage—(*a*) Two inches above the floor—infection takes place; (*b*) one foot above the floor—no infection; proving that, as the animals are at a distance higher than a flea could jump, they have escaped infection.

5. Plague-infected guinea-pigs, with fleas on them, are placed on the floor—
(*a*) One cage with a monkey is surrounded by an area of sticky material broader than the jump of a flea—no infection takes place;
(*b*) Another cage with a monkey without the surrounding sticky material —infection takes place.

6. Healthy rats allowed to feed on the carcasses of infected rats—infection may take place.

7. Plague-infected guinea-pigs allowed to suckle their young ones—no infection takes place.

All these experiments show that rat-flea is the agent which transmits the disease to the healthy. This is also the only method responsible for the transmission of the disease in nature, from one rat to another, and also from rat to man.

Pathogenicity.—Infection always takes place through the skin, generally through some abrasions which may be extremely minute, and through the respiratory tract in cases of pneumonic plague. The commonest source is the regurgitation of the infected blood from the proventriculus of the rat-flea which often becomes completely blocked by a mass of B. pestis. Puncture-wounds, due to flea-bite, may also be infected with the excreta of the flea. Infection also takes place by direct contamination through abraded skin during a post mortem examination.

Pathology of Plague.—Plague is generally evidenced by inflammatory swelling of the deep lymphatic glands—commonly called *bubonic plague.* This is often accompanied by septicæmia. In other instances, there may be primary septicæmia with no recognisable primary bubo. Primary bubonic or primary septicæmic plague may be complicated by secondary pneumonia. There may be, fortunately rarely, *primary pneumonic* form with secondary septicæmia. This type is uniformly fatal. In septicæmic cases, the organisms are found also in the urine.

Mode of Infection and Initial Lesion.—The mode is usually through the skin by the bite of rat-flea. Direct inoculations during post mortem examination or in the laboratory are not uncommon. The initial lesion consists of a tiny pustule at the site of infection, which nearly always escapes observation. The infection extends from the site to the lymphatic gland, producing bubo, or if this line of defence is not competent enough to react, the infection goes to the blood, causing septicæmia. Primary pneumonic plague occurs invariably by infection through inhalation.

The Bubo.—The lymphatic glands show widespread hæmorrhages, foci of necrosis and degenerative changes in the tissues.

The Pneumonia.—B. pestis lodges on the bronchial mucous membrane and then spreads downwards until they reach the infundibulum and air cells. Here they produce lobular consolidation of the lung. In a later stage, one may find fusion of several lobular consolidated areas to one another, forming the type of lobar pneumonia. The patient rarely survives to get the stage of gray hepatisation. The bronchial glands at the bifurcation of trachea also show acute inflammation. Histologically, it is characterised by the absence of fibrin in the alveolus. The sputum is liquid, frothy and pink in colour.

BACILLUS PERTUSSIS

(Bordet & Gengou 1906)

Source.—Expectoration from patients suffering from whooping cough.

Morphology.—Slender rod, non-motile, no flagella, non-sporing, no capsule.

Biology.—Ærobic and grows at blood heat.

Staining Reaction.—Stains well with *aniline* dyes, and is Gram-negative.

Cultural and Biological Reactions.—AGAR and BROTH.—No growth except in subculture. BLOOD AGAR.—Thick, whitish growth with hæmolysis.

Agglutination.—The organisms are agglutinated feebly by the serum of the patient but always gives the complement fixation reaction with the serum, using B. pertussis as the antigen.

Pathogenicity.—Causes whooping cough.

BACILLUS MALLEI

(Lœffler and Schutz 1882)

Source.—Glanders of horse, cattle, etc., which produces an inflammatory condition of the nasal mucosa and upper respiratory tract and also the skin nodules in chronic cases (*farcy*).

Morphology.—Fine rods, measuring 2-5 μ in length. They are non-motile and possess no spore and no flagella. In pus they are often found within the leucocytes.

Biology.—Ærobic and grows best at 37.5°C.

Staining Reaction.—Stains well with aniline dyes, but is Gram-negative.

Cultural and Biological Reaction.—GLYCERINE AGAR.—Colonies are translucent and slimy, becoming brownish and opaque with age. BLOOD AGAR.—Good growth. POTATO.—Same as glycerine agar. No growth within three days.

Agglutination Reaction.—The blood serum possesses specific immune properties.

Pathogenicity.—B. mallei generally affects the horse, causing glanders. Man also acquires the infection through the horse, usually in the hand where a nodule develops in 2-6 days. The neighbouring bony tissue is affected early, but, sooner or later, localises in the lung.

Mallein.—B. mallei produces no true exotoxin. An endotoxin can be easily obtained, which is known as *mallein*. Mallein is extremely resistant and can withstand a temperature of 120°C.,

Mallein Test.—Mallein can be used for diagnosis of glanders. It is diluted 1 in 10 and injected subcutaneously or dropped into the eye directly. Infected horses show a characteristic conjunctivitis. Large amounts of mallein can be injected into healthy animals without effect, but a very small amount will show marked reaction in animals suffering from glanders.

KOCH-WEEK'S BACILLUS

(Koch 1883)

Source.—Acute conjunctivitis.

Morphology.—Slender organism measuring 1-2μ in length, occurring singly or in pairs inside or outside the pus cells. It is non-motile, non-sporing, has no capsule and no flagellum.

Staining Reaction.—Stains well with ordinary stains, and is Gram-negative.

Culture.—AGAR and BROTH—not suitable. BLOOD AGAR—small, transparent colonies.

Inoculation Reaction.—Non-pathogenic to laboratory animals.

Pathogenicity.—Conjunctivitis.

MORAX-AXENFELD DIPLO-BACILLUS

Source.—Chronic catarrhal conjunctivitis.

This bacillus is more or less identical with the Koch-Week's Bacillus.

BACILLUS OF CHANCROID

(Ducrey 1889)

Source.—Genital sores.

These are short bacilli, occurring in chains. They show bipolar staining; growth takes place in blood agar.

PNEUMOBACILLUS

(Friedlander 1882)

Source.—Expectoration from certain pneumonia cases.

Morphology.—Short, thick bacillus surrounded by a capsule. It is non-motile, non-sporing, and possesses no flagella.

Staining.—Stains well with aniline dyes, and is Gram-negative.

Biology and Culture.—It is ærobic and facultative anærobic—grows on ordinary media, but, preferably, in blood agar forming thick, tenacious cream-coloured growth. It produces indol.

Inoculation.—White mice is suitable, with fatal result within 24 to 48 hours.

Pathogenicity.—Causes about 5% of pneumonia.

INTESTINAL GROUP OF ORGANISMS

They are typically intestinal parasites of man and the higher animals, although several of them may occur on plants or in the soil.

They are Gram negative, non-sporing rods, often motile with peritrichate flagella; some species are capsulated. They are ærobes and facultative anærobes and can be easily cultivated on ordinary laboratory media.

The typical form is that of a bacillus 2-3 μ in length and 0.6 μ in breadth with parallel sides and rounded ends. Some are almost coccal in form, others show long filamentous bacilli.

Biochemical Activities.—The biochemical properties form an important means for differentiating the various bacteria of this group. The presence or absence of the power to ferment lactose forms the most important line of clevage within this group. The *lactose fermenters* are for the most part the normal inhabitants of the intestinal tract of man or higher animals or found to exist on various plants or soil. The *non-lactose fermenters* comprise the pathogenic species producing intestinal infections in man or animals. The broad groups are as follows:—

I. Lactose fermenters including B. coli and its allied organisms.

II. Non-lactose fermenters divisible into (*a*) non-gas producers including typhoid and dysentery bacilli, and (*b*) gas producers.

Lactose fermenters—The lactose fermenting coliform bacilli have been subdivided into two primary divisions on the basis of $CO_2:H_2$ ratio, Voges Proskauer reaction and methyl red test.

1. The *first group* contains strains which gives $CO_2:H_2$ ratio as 2:1, V-P reaction positive and methyl red test negative. The great majority are isolated from plants, grain, unpolluted soil and water.

2. The *second group* contains strains which give a $CO_2:H_2$ ratio of approximately 1:1, V-P-R-negative, methyl red test positive. They comprise the great majority of strains isolated from intestines of man or animals. Their presence in water denotes fæcal contamination.

Bacteria which are found in Water are—

(1) Bacteria derived from the surface layers of the soil; *e.g.*, (*i*) B. fluorescens; (*ii*) chromogenic bacteria, *e.g.*, B. Prodigiosus, B. ruber, B. indicans forming red pigments; B. aquatilis, B. aurantiacus and B. fulvus forming orange or yellow colonies; B. violaceus and B. cæruleus forming violet pigment; (*iii*) proteus group; (*iv*) sporing bacteria, *e.g.*, B. subtilis, B. mycoides, B. mesentericus; (*v*) cocci, *e.g.*, M. candicans, M. aquatilis; and (*vi*) organisms not belonging to the above groups.

(2) Fæcal organisms polluting the water; (*i*) coli group, *e.g.*, B. coli communis, B. coli communior, B. acidi lactici, and B. neapolitans; (*ii*) ærogenes group; *e.g.*, B. lactis ærogenes, and B. cloacæ; (*iii*) fæcal streptococcus; (*iv*) fæcal anærobes, *e.g.*, B. welchii and B. sporogenes.

The intestinal group of organisms, also called the *coli-typhoid group*, is divided into three sub-groups. They are as follows:—

(*a*) The Colon Group—B. coli. communis, B. lactis ærogenes, B. acidi lactici.

(*b*) The Gærtner Group—

(*i*) B. enteritidis (Gærtner's bacillus);

(*ii*) B. paratyphosus A;

(*iii*) B. paratyphosus B.

(*c*) The Typhoid Group—

(*i*) B. typhosus;

(*ii*) B. dysenteriæ (B. shiga, B. flexner, etc.).

Characters of the Three Groups:—

	Colon Group.	Gærtner Group.	Typhoid Group.
Lactose bouillon ..	Gas and acid.	No change.	No change.
Glucose bouillon ..	Gas and acid.	Gas and acid	No gas but acid.
Neutral red ..	Reduced.	Reduced.	No reduction.

The above table shows that three substances are used for the group-tests. All these substances indicate change with the colon group and just the opposite of the typhoid group. The Gærtner Group stands intermediate.

BACILLUS COLI (ESCHERICHIA COLI)

(Escherich 1886)

(*Colon Bacillus or B. coli communis*)

Source.—Normally present in human intestines. In pathological conditions in urine, blood, gall bladder, etc. Also present in water contaminated with sewage or fæcal matter.

Morphology.—Short, plump, with rounded ends, measuring 2-3 μ long. Involution forms, as well as coccoid, shorter or longer forms, are common. It is motile, possesses 3 or 4 flagella, but no spore and no capsule. Certain strains of B. coli are definitely encapsulated and free from motility.

Biology.—The B. coli is an ærobe and a facultative anærobe and grows at body temperature (37·5°C).

Staining Reaction.—Stains readily with aniline dyes, and is Gram-negative.

Cultural Reactions.—Agar.—Thick, moist, shining cream-coloured growth. Broth.—Turbidity with production of indol. There is a heavy sediment but no pellicle. Gelatin.—Growth similar to that in agar. Gelatin is not liquefied. Potato.—Moist, brownish yellow, thick growth. MILK.—Acid (lactic) and coagulation. PEPTONE WATER.—Turbidity with production of indol.

B. coli decomposes proteins, with production of a number of substances, the most important of which are indol, phenol, hydrogen sulphide, etc. [*Test for Indol.*—Add 1 c.c. of 0·02% potassium nitrite solution and a few drops of concentrated H_2SO_4 into a 24-hour culture. A rose or a deep-red colour indicates indol.] Nitrates are reduced to nitrites by B. coli, but it is better to add nitrite. (Cf. also cholera-red reaction.—*See* cholera vibrio.)

Smooth and Rough Colonies.—From the appearance of the margin two different types of the organisms are differentiated. Those with smooth outline are called ***smooth*** (S), and those with irregular outline are called ***rough*** (R) colonies. "S" colonies possess a smooth outline, are round, raised, shiny and transparent, while "R" colonies are irregularly outlined, flat, slightly opaque and have a dull surface.

"S" and "R" colonies are also recognised with all the organisms belonging to the typho-coli groups.

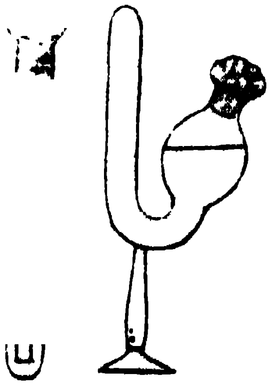


Fig. 54. Fermentation tubes for collection of gas.

Sugar Fermentation.—It has been found that pathogenic strains isolated from urinary tract infections are slow lactose fermenters. All the sugars are fermented with production of acid (lactic) and gas (carbon dioxide and hydrogen).

Neutral red in glucose broth.—Flourescent.

Hæmolysis.—Some strains of B. coli hæmolyses blood.

Keyser found a heat stable lysin in cultures of B. coli, and prepared an anti-hæmolysin. Schmidt found hæmolytic strains more frequently present in the inflamed than in the healthy intestines.

Thus, the special cultural reactions are:—

(*a*) Fermentation of sugar, with production of acid and gas.

(*b*) Curdling of milk and production of permanent acidity.

(*c*) Production of indol.

(*d*) Decomposition of proteins into nitrates.

(*e*) Flourescence in neutral red bouillon.

Special cultures in MacConkey's medium, Conradi-Drigalski medium, etc.:—In MACCONKEY—colonies are red (fermentation of lactose). This, as well as other special media are used to differentiate B. coli from other intestinal organisms. There are different varieties of B. coli. some typical, others atypical. *The Characters of a Typical B. coli are as follows*:—Houston abbreviates them by the term "Flaginac."

Fl—Flourescence in neutral red glucose broth.
Ag—Acid and gas in sugars.
In—Indol in peptone water.
Ac—Acid and coagulation of milk.

The Typical B. coli does not give the *Voges-Proskauer reaction* (in which a red fluorescence is produced after about 24 hours if caustic potash solution is added to a three-day culture of glucose peptone).

Agglutination Reaction.—B. coli is agglutinated by the specific serum, but it may also be agglutinated with one obtained with another variety occasionally, even with normal serum.

Inoculation Reaction.—Intraperitoneal or intravenous administration into a rabbit or guinea-pig produces septicæmia, occasionally localised inflammatory changes. Subcutaneous inoculation in laboratory animals is usually followed by formation of an abscess.

Pathogenicity.—Usually non-pathogenic. It produces commonly cystitis and cholecystitis, and positive culture may be obtained from the blood. When pathogenic, it produces septicæmia, pyæmia, ulcerative endocarditis, peritonitis, suppurative affection of the appendix and gall bladder, puerperal fever, pneumonia, cystitis, pyelitis, and occasionally leads to the terminal infection.

In man, B. coli appears at a very early stage in the infant's intestines and throughout life, man carries millions of these organisms in colon. In health, B. coli is confined to the large intestines or at any rate, does not extend far beyond the ilio-cæcal valve into the small intestines. One of the commonest conditions in which infection with B. coli occurs in man is cystitis and in this disease it is said that if blood cultures are made during a rigor, B. coli is frequently isolated.

Mode of Infection.—B. coli is confined to the intestines in health, but often infect other organs mainly by the following three routes: (*i*) directly from the intestines to the infected area, (*ii*) by the lymphatics, and (*iii*) by the blood stream.

BACILLUS TYPHOSUS (EBERTHELLA TYPHI)

(Eberth 1880, Gaffky 1884)

Source.—(1) Blood, fæces, urine and rose spots on the skin (abdomen). (2) Mesenteric glands, spleen, intestines (Peyer's patches), gall-bladder and liver.

Morphology, Biology and Staining.—Same as B. coli, except that they are longer, thinner and more motile than B. coli, and that the flagella are longer and larger in number than B. coli (8-12) and they are arranged peripherally about the body of the organism. They are ærobes and facultative anærobes.

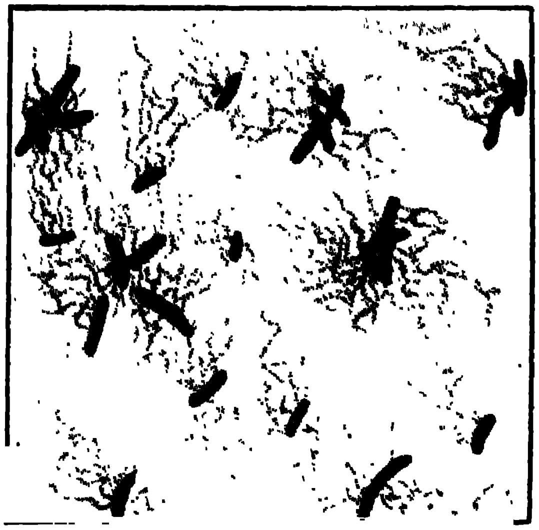


Fig. 55. B. typhosus showing flagella.

Cultural Reactions.—AGAR.—Moist, bluish-grey growth with regular margins in some (called " smooth " colonies), while irregular in others (called " rough " colonies). BROTH.—Uniform turbidity. POTATO.—Growth moist, shining and invisible, especially when the potato is fresh and slightly acid in reaction.

Biological Products.—These are quite distinct from those of B. coli. They are as follows:—

(1) Milk.—Acid but no coagulation. (2) Indol is absent. (3) Fermentation of sugar:—(*a*) Glucose and maltose—only acid, no gas. (*b*) Lactose—not fermented. (4) Agar culture washed free from the organism with saline is unsuitable for further growth of B. typhosus. (5) MacConkey plate--colonies are colourless, *i.e.*, no fermentation of lactose.

Inoculation.—Laboratory cultures are often harmless; feeding, or subcutaneous or intraperitoneal injection is often unsuccessful. If any pathogenic effect is produced, it produces a toxæmia or septicæmia in laboratory animals, and never resembles the disease occurring naturally in man. In nature no animal other than man is attacked by true typhoid.

Agglutination.—There is a strong agglutination reaction with specific serum. The reaction is obtained after the first week of fever.

Organisms may be agglutinated with normal serum in 1 in 20 dilution, but agglutination with serum in a dilution of 1 in 100 indicates that—

(*a*) The patient is suffering from an attack of typhoid fever; or
(*b*) He is a typhoid carrier; or
(*c*) He is convalescent from typhoid fever; or
(*d*) He has recently been inoculated with anti-typhoid vaccine.

The agglutination reaction appears, generally, after the tenth day of illness, but may not appear until during convalescence. It generally disappears within a year.

Pathogenicity.—It causes enteric fever. So far as is known, no other animal, other than man, suffers from true typhoid fever.

Mode of Spread.—B. typhosus generally enters through the alimentary tract and passes through the stomach when the acid content is low, either by disease or mechanically by large draught of water, etc. Multiplication takes place in the alkaline intestinal content containing bile. The organisms enter through the Peyer's patches or solitary follicles into the lymph spaces, mesenteric glands and spleen. This takes place during the incubation period and gives rise to vague symptoms of headache and malaise. From the spleen they pass through the lymph channels, and the thoracic duct into the systemic circulation and are thus carried to all the organs and are voided with the urine during the second week or beginning of the third week of the fever. Many are at once destroyed, toxins are set free and give rise to the initial stage of fever. During the second week, they pass through the liver into the gall bladder and again into the intestines. Vast numbers of the organisms are destroyed in the circulating blood, with liberation of toxin and thus causing the symptoms.

The above mode of spread is proved by the fact that, in nearly 100% of cases, B. typhosus can be isolated from the blood during the first week of fever, and, in a later period, from the fæces, and later still, from the urine.

The toxins are mainly intracellular, and inoculation in lower animals produces septicæmia without producing the characteristic intestinal ulcerations as seen in man.

Anti-typhoid Inoculation.—Wright's method is usually employed. This consists of injection subcutaneously of two doses at intervals of 10 days. First dose--500 million organisms, 2nd dose—double the first dose.

In the military services, a triple vaccine known as T. A. B. vaccine (Typhoid, para A. and para B. vaccines) is used. 1 c.c. of T. A. B. vaccine contains—B. typhosus, 1,000 millions; B. para A., 500 millions; and B. para B., 500 millions. Three doses are used at intervals of one week: First dose—0·5 c.c., second and third doses—1 c.c. each.

Carriers.—After an attack, certain individuals remain carriers for varying periods—even for several years. Boldnan and Noble isolated B. typhosus 46 years, and Gregg 52 years, after the original attack. It is said that all individuals who remain as carriers for 3 months, remain so for the rest of the life. The carriers harbour the organisms in the gall bladder or in the urinary tract.

BACILLUS DYSENTERIÆ (EBERTHELLA DYSENTERIÆ)

There are several varieties, of which the following are commonly distinguished:—(1) B. shiga (Shiga 1889); (2) B. flexner; (3) Sonne type; (4) Schmitz type; (5) Paradysentery bacilli. These are found rarely in dysenteric stools. Y and Strong types have been classified as special types of the Flexner group.

They resemble one another closely with slight differences. The points of difference lie mainly in sugar reaction and production of toxin *e.g.*:—*Shiga type*:—(*a*) Produces no acid in mannite medium. (*b*) Produces powerful toxin in culture. *Flexner type*:—(*a*) Produces no acid in mannite medium. (*b*) Produces feeble toxin in culture.

The common characters are the following:—

Source.—From fæces of a patient suffering from bacillary dysentery. When the dysentery bacilli tend to disappear from the fæces, certain other organisms, called "concomitants," are found to be present in the material. These organisms are (*i*) B. Morgan No. 1, (*ii*) B. fæcalis alkaligenes, and (*iii*) B. paracolon.

Morphology.—They resemble B. typhosus but are slightly thicker. They are non-motile, possess no flagella, no spore formation, no capsule; involution forms occur in culture.

Biology.—They are ærobes and grow best at 37°C.

Staining Reaction.—They stain well with aniline dyes, and are Gram-negative.

Cultural Characters.—AGAR.—Smooth, moist, cream coloured growth. BOUILLON.—Uniform turbidity. POTATO..—At first, whitish, then brownish growth.

Biological Products.—SUGAR.—No gas is produced in any sugar by any organism of this group. B. flexner ferments mannite, which is not fermented by B. shiga. For other sugars, *see* the following chart.

Chart showing Sugar Reactions of the Dysentery Bacilli

	Shiga.	Flexner.	Strong.	"Y"
Mannite ..	—	A	A	A
Maltose	—	A	—	—
Saccharose ..	—	—	A	—

None ferments lactose. All ferment glucose with production of acid. MILK.—Temporary acidity followed by alkalinity with no coagulation. INDOL.—This is produced by B. flexner and not by B. shiga.

Agglutination Reactions.—The organisms are agglutinated by specific sera. Agglutination phenomena are observed in blood generally during convalescene. An agglutination of 1 to 50 dilution indicates a Shiga infection and one of 1 to 150 a Flexner infection

Animal Inoculation.—B. shiga is very virulent to ordinary laboratory animals.

Toxins.—The Shiga bacillus produces an endotoxin and an exotoxin. The filtrate of Flexner culture is almost harmless when compared to that of Shiga. Flexner possesses an endotoxin but no exotoxin.

Pathogenicity.—Bacillary Dysentery.—*Mode of Spread.*—The Shiga bacillus enters through food, and one may notice the first symptoms within 12 hours. The constitutional symptoms and the intestinal lesions are the result of a powerful toxic action and not by invasion of the blood. It is mainly a toxæmia.

Dysenteric arthritis, pyæmic symptoms may develop as complications. Chronic dysentery is common with the Shiga Bacillus and less frequent with the Flexner Bacillus.

Paradysentery Bacillus.—Morphologically and culturally, these are all members of typho-coli group of organisms. They lie between B. coli and dysentery in characters. These are Morgan (No. 1) bacillus, paradysentery bacillus of Lœwenthal (1912), paradysentery bacillus of Negre, Sergent and

Foley (1916), Morgan and Ledingham groups (1909), Douglas and Colebrook (1917), B. coli anærogenes, etc.

Cytological Examination of Dysenteric Stools.—

A definite picture of the pathological changes in the walls of the intestines can be obtained by cytological examinations of the stools.

(*i*) *During the first* 24 *hours* the material passed per rectum consists of relatively clear mucus streaked with blood or containing groups of red blood cells. This is due to the intense congestion of the mucous membrane of the large intestines with large quantity of secretion of mucus.

(*ii*) *During the next* 24 *hours* evacuation consists of bright red blood, semiopaque mucus and a clear fluid. Microscopically, one finds a large number of red blood cells, pus cells and epithelial cells. Macrophages may also be found.

(*iii*) *From the second to the fourth day,* stools consist of semiopaque mucus. Bright red blood is no longer the prominent feature. Microscopically, the sample of stool consists entirely of pus cells.

(*iv*) *About the fifth day,* little or no blood is seen and the mucus appears purulent. Microscopically more than 90% of cells are pus cells. At this period, coagulation necrosis and ulceration of the mucous membrane is taking place.

(*v*) *After the fifth day,* depending on the severity of the case, fæcal matter appears in the stools.

Serology of the salmonella group:—This can be grouped under two heads.—

"H" antigen and "O" antigen.—The typical organism belonging to the salmonella group contains two antigenic constituents concerned in the agglutination reaction. The "H" agglutinogen probably represents, in part, the flagellar portion and it is almost invariably associated with the motile phase. By shaking and then centrifugalising, the bodies of the bacilli are thrown down and the flagella are left in the clear supernatant fluid. Injection of this fluid or the whole bacilli, produces a floccular agglutinating serum, whereas the injection of the bodies of the organisms alone produces a granular agglutinating serum—("O" antigen). When the supernatant fluid is heated to 70°C or over, it can be demonstrated that the flagella are destroyed and the fluid no longer flocculates. On growing B. typhosus on carbolised media it was found to be without flagella and no "H" or floccular agglutinins or agglutinogens could be demonstrated. The serum of animals inoculated with emulsions from this culture showed only granular agglutinins. Thus the typical salmonella contains two distinct classes of antigens.

The " H " antigen, or coarsely flocculating antigen, is thermolabile, more or less severly damaged at temperature above 65°C and when heated at 100°C or when treated with alcohol or dilute acid are totally destroyed ceasing to function in serological tests or as antigens. Their development is inhibited by culture on certain media, notably, phenol agar medium. As has been noted above, this antigen is derived from flagella.

The " O " antigen, or the finely granular antigen, resists prolonged heating at 100°C and also treatment with alcohol and dilute acids. They are unaffected by phenol agar culture. This antigen is derived from the body of the bacilli.

The flagellar antigen plays little, if any, part in active immunisation. The somatic antigen is solely responsible for the phenomenon in complement fixation in presence of specific serum. Some believe that both the somatic and flagellar antibodies exert a marked effect in forwarding phagocytosis.

Laboratory diagnosis of typhoid and paratyphoid infections.—

1. BLOOD CULTURE.—This is successful in the early stage only. The chance of success diminishes rapidly after the first week of illness. (a) 1 ccm. of patient's blood inoculated into a tube containing about 4 ccm. of sterile ox bile and incubated for 24 hours to 5 or 6 days. (b) 5 ccm. of the patient's blood inoculated into 50 ccm. of glucose broth solution and incubated.

2 ISOLATION OF THE ORGANISMS FROM FÆCES.—This is a very uncertain method especially in mild cases and in individuals protected by inoculation. The elimination of bacilli through fæces may be very transient. The routine culture is one in MacConkey, Endo, Conradi-Drigalski or similar differential media. The addition of brilliant green (1|1, 50,000) with telluric acid (1|15,000) has been found to be of great help with certain workers, as it has been found that when present in a certain concentration, it has a selective action on typhoid and paratyphoid organisms while inhibiting the development of coliform bacilli.

3. ISOLATION OF THE ORGANISMS FROM URINE.—The media used are the same as those of fæces. This should be undertaken during the second and third weeks of illness.

4. SEROLOGICAL EXAMINATION OF BLOOD (Agglutination tests.—) This should be undertaken from the second week.

BACILLUS (SALMONELLA) PARATYPHOSUS (A & B)

(Achard & Bensaude 1896)

There are two varieties of paratyphoid bacillus known as A and B, to which is added a third species, paratyphosus C, isolated by Uhlenhuth and Huebner in pig (1909), and subsequently found also in human fæces. They are organisms intermediate in type between the bacillus typhosus and B. coli. Of these organisms, Para B is commoner than Para A, as causative factors of diseases (Para B occurring in 80% and Para A in 20% of cases). The case mortality is higher in paratyphoid B infection than in A. The B. paratyphosus B has also been found to cause food poisoning and acute gastroenteritis.

Source.—Same as B. typhosus.

Morphology.—etc.—Same as B. typhosus.

Difference between Para A and Para B

	Para A.	Para B.
Lead acetate agar* ..	No change of medium.	Blackening of the medium.
Litmus milk ..	Permanent acidity.	Temporary acidity followed by alkalinity.
Neteral red ..	Turned yellow temporarily for 2 or 3 weeks.	Turned yellow permanently.
Agglutination ..	Agglutination by specific Para A serum.	Agglutination by specific Para B serum.

B. ENTERITIDIS

(Gærtner 1888)

Source.—Meat in case of meat poisoning.

The organism resembles B. paratyphosus, and can be distinguished only by the agglutination reactions.

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* Lead acetate agar contains—Nutrient agar, 100 parts; Dextrose, 1 part and Lead acetate, 0.1 part.

Table showing the Characters of the Organisms of the Coli-typhoid Group

Organism.	Motility.	Glucose.	Lactose.	Mannite	Indol.	Litmus
B. coli ..	+	A. G.	A. G.	A. G.	+	A. C.
B. typhosus ..	+	A.	—	A.	—	A.
B. paratyphosus A.	+	A. G	—	A. G.	—	A.
B. paratyphosus B.	+	A. G.	—	A. G.	—	A. Alk.
B. enteritidis Gærtner	+	A. G.	—	A. G	—	A. Alk.
B. shiga ..	—	A.	—	—	—	A. Alk.
B. flexner ..	—	A	—	A.	+	A. Alk.

+ indicates presence of motility or formation of indol as the case may be.

— indicates absence of motility or formation of indol or absence of any change

A. production of acid.
G. production of gas
Alk. production of alkalinity.
C. production of clot.

SPIRILLUM (VIBRIO) CHOLERÆ (VIBRIO COMMA)

(Koch 1883)

Also known as Comma Bacillus, or Cholera Bacillus. Strictly speaking, this organism belongs to the family of Spirilla. It is described here for the sake of convenience.

Source.—Evacuations from bowels of cholera patient. Contents of gall-bladder, mucous membrane of intestine after death from cholera.

Morphology.—Small curved organisms, measuring 1.5-2μ in length, and 0.5μ in breadth. They occur singly, often in pairs, and are exceedingly pleomorphic. When single, they have an appearance like a comma (,). When in pairs, they often attach themselves in opposite directions, so as to form the appearance of the letter " S." Occasionally, several of them are attached to one another, forming a spiral filament. When examined from fresh material from intestines, they are found in large numbers, with their long axes lying in the same direction—*the so-called fish-stream appearance.* They are very actively motile, and possess a single flagellum at one end. They do not form spores, there is no capsule. In old cultures, involution forms are very common.

Biology.—The organisms are ærobic and facultative anærobic, and grow well at 37.5°C.

Staining Reactions.—They are stained readily with aniline dyes, and are Gram-negative.

Cultivation.—They grow readily in ordinary media. AGAR.—Thin, moist growth, more bluish than B. coli colonies. TAUROCHOLATE AGAR (agar containing 0.5% sodium taurocholate and with pH 7.6 to 9·0).—Grows luxuriantly. ALKALINE BOUILLON.—Uniform turbidity with formation of a pellicle on the surface which consists of actively motile vibrio. GELATIN.—Whitish colonies with subsequent funnelshaped liquefaction of gelatin. SOLIDIFIED BLOOD SERUM.—Thin growth with liquefaction of the media. ALKALINE POTATO.—A brownish moist layer. It has been found that growth occurs freely under anærobic conditions in the presence of fermentating sugars. This is taken to explain the capacity of V. cholera to multiply abundantly in the bowels and liberate toxins.

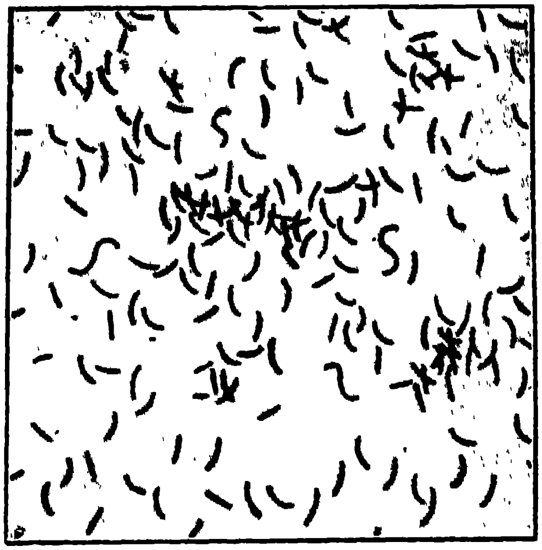

Fig. 56 V. Choleræ
Smear from broth culture.

Biological Products—MILK.—No coagulation.

Sugar Reactions.—Ferments glucose with production of acid, but no gas. It is a late lactose fermenter, *i.e.*, lactose is fermented after 2-3 days with production of acid and no gas. INDOL and NITRITES.—It produces indol and nitrates are reduced to nitrites. For this purpose, either bouillon or alkaline peptone water is used. The organisms are allowed to grow for 24 hours at 37·5°C. A few drops of pure sulphuric acid is added to the culture—a reddish pink colour is produced, the so-called "Cholera-red Reaction." The substance giving the red colour is nitroso-indol. H_2SO_4 combines with the nitrite to give the pink colour.

Hæmolysis.—Many true cholera vibrios do not produce any hæmolysis but El Tor group of organisms (Pseudo-cholera organisms) produce hæmolysis. The apparent hæmolysis produced by the cholera vibrio is really due to hæmodigestion. Hæmolysis is best observed in alkaline bouillon.

Special Culture Media.—This depends on the fact that cholera vibrio grows abundantly in an alkaline medium which will inhibit the growth of other organisms:—

(1) *Durham's Peptone Water.*— In this medium, cholera vibrio grows on the superficial layers, and much more rapidly than the other intestinal organisms which grow in deeper layers. On the surface, a pellicle is formed, which is composed of actively motile cholera vibrio. The growth often takes place within six hours.

(2) *Dieudonne's Medium* (*Alkaline blood agar*).—In this medium no other organisms, including those of the coli-typhoid group, will grow except the cholera vibrio. This is due to the alkalinity of the medium.

(3) *Maitra and Basu's Taurocholate Agar Medium.*—Grows well.

Agglutination Reaction.—The organisms are agglutinated by specific serum.

The biological reactions as given by the vibrio are not sufficient to establish the positive isolation of the organisms. Serological tests are necessary to establish the positive diagnosis. These are:—

1. Bacteriolysis as evidenced by the Pfeiffer's reaction.
2. Complement fixation test.
3. Agglutination reactions. The most important and at the same time the easiest methods are:—(*i*) Rapid slide method—the organisms from a colony or from pure culture on slope agar are suspended on a slide in low dilution of high titre serum (1 in 100), (*ii*) Dreyer's method, (*iii*) microscopical method.

The subject of agglutination reaction has recently become very complex owing to the discovery of several factors, *e.g.*, bacterial variation, smooth-rough type variation, specific-non-specific phase variation etc. Besides these a great controversy has grown up about the question whether an agglutinating cholera vibrio can change into a non-agglutinating type and *vice versa.* There are strong arguments both for and against this question.

Bacteriolysis.—This has been described by Pfeiffer and is known as the Pfeiffer's phenomenon. This really represents a mixture of bacteriolysis and agglutination *in vivo.*

Resistance.—The cholera vibrio is very susceptible to high temperature, sunlight, drying and to the growth of other organisms.

Toxins.—The organism has an endotoxin. Some schools believe in the existence of a soluble exotoxin, but this is not accepted.

Animal Inoculation.—Animals are not infected naturally with cholera vibrio. Intraperitoneal injection in guinea-pig leads to infection with peritonitis. If the dose is large, they may appear in the blood. Injection, into the ear vein of a young rabbit, of a few drops of a 1 in 1,000,000 dilution of a pure culture leads to a characteristic diarrhœa terminating in death. Pigeons are almost immune.

Pathogenicity.—It causes cholera. It enters by the mouth through food or water. If not killed by the acid gastric juice, it enters into the alkaline intestinal juice which acts as a favourable culture medium and produces the lesions. The superficial layer of the mucous membrane of the small intestine is desquamated, which, mixed up with the contents, appears as flakes in the evacuation which takes the appearance of rice water due to the absence of bile, the turbidity being due to the flakes of epithelium.

The evacuations become very frequent and in great bulk, coming out in the form of both vomiting and purging. A great loss of fluid from the body takes place early, resulting in the rise of the specific gravity of blood, cramps, retention of urine and, ultimately, collapse due to fall of blood pressure. The kidney is damaged early in the form of degenerative changes of the tubules, which often leads to the dangerous complication uræmia, and finally death. On post mortem examination, marked dehydration of the tissues is observed, with superficial necrosis of the mucous membrane of the small intestine. The gall bladder is nearly always distended with dark-green thick bile.

Paracholera and Cholera-like Vibrios

These are:—1. El Tor vibrio. 2. Metchnikoff's spirillum. (3) Finkler and Prior's spirillum. They are differentiated by agglutination reactions.

CHROMOGENIC BACILLI

BACILLUS PYOCYANEUS

(Gessard 1882)

Source.—Abscess cavities, respiratory tract, otitis media, fæces.

Morphology.—They are minute rod-shaped organisms measuring 2-5 μ in length. They are motile, possess a single polar flagellum no spores, and no capsule.

Biology.—They are ærobes, and grow best at 37.5°C.

Staining Characters.—They are stained readily with aniline dyes, and are Gram-negative.

Cultivation and Biological Products.—They grow readily in ordinary media, with the production of a pigment which varies in tint from a bright blue to green or greenish-brown colour. There are two pigments—(1) a bright-red, non-fluorescent pigment, soluble in water (*pyorubrin*), and (2) a non-fluorescent blue pigment soluble in chloroform (*pyocyanin*).

The colour diffuses through the medium and can be dissolved by putting a few drops of chloroform in the culture media, the chloroform becomes blue. Gelatin is rapidly liquefied.

Pathogenicity.—It causes "blue pus," sometimes met with in abscesses. It is believed that it is only a secondary infection in these lesions. It is frequently found in old wounds, otitis media, and discharging sinuses.

Toxin.—It forms a soluble exotoxin in addition to the endotoxin.

BACILLUS PRODIGIOSUS

It grows very luxuriantly in artificial media, with the production of a brilliant red pigment when placed in room temperature. The pigment may be absent when the organisms are allowed to grow in the incubator.

BACILLUS VIOLACEUS

It produces a violet pigment.

CHAPTER XI

SPIRILLA AND SPIROCHÆTES

The true nature of this group of organisms is not definitely known. It is generally held that Spirilla and Spirochæte are different organisms. The difference lies in the following:—

Spirilla are rigid, and the spirals are preformed.

Spirochætes are flexible, and the spirals are not preformed. The next difficulty is as regards the real position of spirochætes in biology. There is a good deal of discussion as to whether it is an animal or a vegetable organism; or, in other words whether it belongs to *Protozoa* or *Bacteria.* However, instead of going into any details, we may, for the present, group it under *Bacteria* and not under *Protozoa.*

The following might be mentioned as the differentiating points:—

For Bacteria.	*For Protozoa.*
1. Absence of nuclear structure.	1. Possession of undulating membrane (only in a few).
2. Transverse division.	2. Difficulty of cultivation in artificial media.
3. Production of active immunity	

Spirochœta (Ehrenberg 1834).

It is fairly large in size, measuring up to $500\mu \times 0.75\mu$. It is cylindrical and possesses regular spirals. There is no crista or flagella but it possesses an elastic flexible axial filament. Type-species—*S. plicatilis,* a free living form.

Cristispira (Gross 1910)

They are organisms which possess axial filaments in the form of a prominent raised ridge known as the crista, hence the name of the group. type-species—*C. balbiandi.*

Leptospira (Noguchi 1917)

The spirochætes have a spiral twist like a rope, in addition to a tendency to curved loops at one or both ends, during movement. Type-species—*L icterohæmorrhagiæ.*

Treponema (Schaudinn 1905). Vide *infra.*

Morphology.—They are long, filamentous, spiral organisms. The cell wall is flexible. There are no nuclei, flagella, or other organs of locomotion. How they move is not definitely known. Dobell is of opinion that the body is composed of numerous compartments separated by transverse partitions, each compartment having a few very fine chromatin dots. In some cases, metachromatic granules are present.

Cultivation.—Noguchi's method.

Multiplication.—Here, again, there is a good deal of controversy. Some authorities, including Noguchi, say that they divide by longitudinal binary fission, but this is doubted by others. Dobell is of opinion that they divide transversely.

Life Cycle.—Not definitely known. It is supposed that the spirochæte breaks up into small granules inside the cell membrane. These granules are the infective agents. These grow up again into spirochætes.

Transmission.—They are transmitted by ticks and lice. In the tick, the organism passes to the cœlomic cavity through the wall of the gut, and thence, to every part of the body. Here they break up into a large number of granules which are infective.

The granules are present in the Malpighian tubules and ovaries. Thus, the intestinal contents as well as the ova are infected, and by this way, infection is transmitted to the next generation. They also infect when crushed on the abraded skin of the host.

Classification.—The scientific classification is difficult. The following clinical classification is more convenient:—

A. Spirochætes inhabiting blood:—

(1) T. pallida—causing syphilis; (2) T. pertenuis—causing yaws; (3) S. obermeieri or S. recurrentis—causing European relapsing fever; (4) S. duttoni—African tick fever; (5) S. novyi—American relapsing fever; (6) S. carteri—Indian relapsing fever; (7) S. berberi—Egyptian relapsing fever; (8) Spirillum minus (S. morsus muris)—rat-bite fever; (9) Leptospira icterohæmorrhagiæ—epidemic jaundice, (10) L. icteroides—yellow fever.

B. Spirochætes inhabiting skin and mucous membranes:—

(1) S. vincenti, along with fusiform bacilli—(*a*) Vincent's angina, (*b*) Naga sore, (*c*) Tropical ulcer; (2) S. refringens—found in genital sores—non-pathogenic; (3) S. bronchialis—causing bronchitis; (4) S. mucosa—normally present in the mouth; (5) S. eugyratum—inhabiting intestines.

C. Spirochætes supposed to cause fevers of unknown origin:—

Since the discovery of the leptospira group causing yellow fever and epidemic jaundice, it has been believed that other diseases with clinical resemblance are also caused by spirochætes. In some instances, the transmitting agent is known, *e.g.*, mosquito and dengue, but the organism is unknown.

The following fevers are supposed to be caused by Spirochætes:—

1. *Dengue.*—Transmitted by stegomyia. Couvy found spirochætes in 1921 in seven-day fever.
2. *Sandfly Fever.*—Whittinghum found leptospira in 1922. Transmitted by phlebotomus. Couvy found spirochætes in 1921.
3. *Rocky Mountain Spotted Fever.*—Transmitted by tick.

Many theories (*a*) Piroplasma. (*b*) Bacteria. (*c*) Rickettsia bodies Rickettsia bodies are characterised by the following:—

(1) Stained by Giemsa and Lœffler's stain but poorly.
(2) Very minute in size—less than 0·5 μ in diameter.
(3) They multiply in the epithelial cells of the insect.

4. *Trench Fever.*—Transmitted by lice. It is not the bite which is infective. The scratches induced by irritation of the pediculæ lead to abrasion and crushing of the body of the lice. The crushed body as well as the fæces infect the abrasions. It is also supposed to be a Rickettsia affection.
5. *Typhus.*—Transmitted by lice. The crushed lice and their fæces infect the abrasion by scratching. Supposed to be caused by Rickettsia prowazekia.
6. *Japanese River Fever* or *Tsutsugamushi Fever.*—Transmitted by a mite.

VIBRIO CHOLERAE (*SEE P.* 166.)

TREPONEMA PALLIDUM

(Schaudinn 1905)

The organism belongs to the genus "Treponema" which is characterised by (*a*) symmetrical, regular spirals, (*b*) ends pointed, (*c*) cultivation and staining with great difficulty, (*d*) no axial filament, no crista or no undulating membrane, (*e*) movement by corkscrew action due to revolution on the longitudinal axis.

The organisms belonging to this genus are:—

T. pallidum (of syphilis), T. pertenuis (yaws), T. recurrentis (relapsing fever), T. dentium (of mouth), T. vincenti (of throat), T. eurygyratum (of intestine), T. schaudinni (of tropical ulcers), T. refringens (of ulcerating surfaces), etc.

T. pallidum was discovered by Schaudinn and Hofmann in 1905. Syphilis has been known to the world for many centuries, but the causative organism has been known only recently. This is due to the fact that the organism persistently refused to grow in any culture medium, and the appropriate staining method was not known. They escaped detection also for the very fineness of the structure.

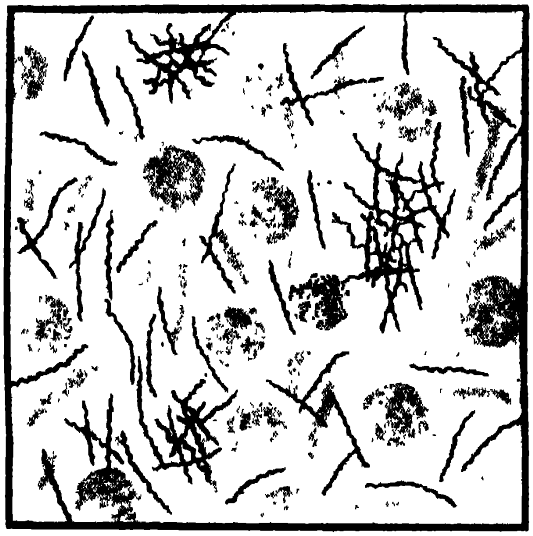

Fig. 57. Treponema pallidum in section from tissue.

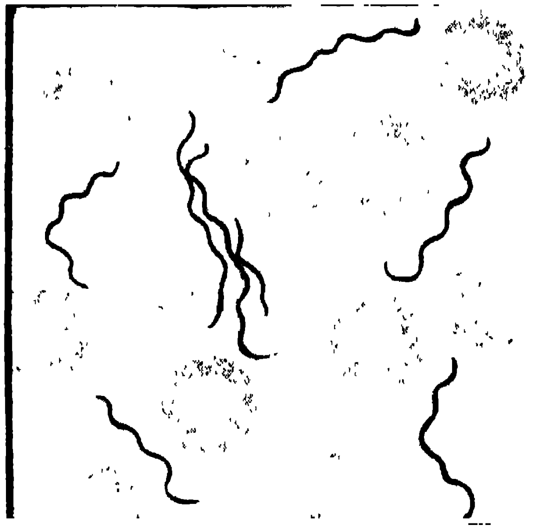

Fig 58. Spirochæta of relapsing fever.

Source.—They are found in all the stages of syphilis:—

(*a*) Very numerous in primary chancre; (*b*) Numerous in the secondary lesions; (*c*) Scanty in the tertiary lesions—generally, at the periphery of the lesion; (*d*) Numerous in the lymphatic channels —glands in the neighbourhood of the chancre; (*e*) Scanty in blood; (*f*) Very scanty in the cerebro-spinal system in cerebro-spinal syphilitic conditions; (*g*) Very numerous in the organs, especially the liver, in cases of congenital syphilis.

Morphology.—Size varying from 8-15 μ in length, with 8-15 regular, fine, symmetrical spirals. For examination of the material, dark ground illumination is the best, which demonstrates clearly the characteristic appearance and movement of the organism. It is actively motile, showing a backward and forward movement, also rotating about the long axis.

Staining.—The organism can be stained by the following methods:—1. Indian ink method.—The organisms remain unstained in black background; 2. Congo-red method; 3. Fontana method; 4. Giemsa staining; 5. Leishman staining.

Cultivation.—Noguchi's method or McLeod's or Soga's modification of same.

Pathogenicity.—This organism causes syphilis. (For details, *see* under *specific granulomata.*)

TREPONEMA PERTENUIS

(Castellani 1905)

Source.—All the lesions of yaws or framboesia.

Morphology.—Same as T. pallidum, with the difference that T. pertenuis is less uniform and more variable in length and has a tendency to round up into ring forms.

Staining and Cultural Reaction.—Same as T. pallidum.

Pathogenicity.—It causes the disease known as yaws. Gangosa is probably one stage of yaws.

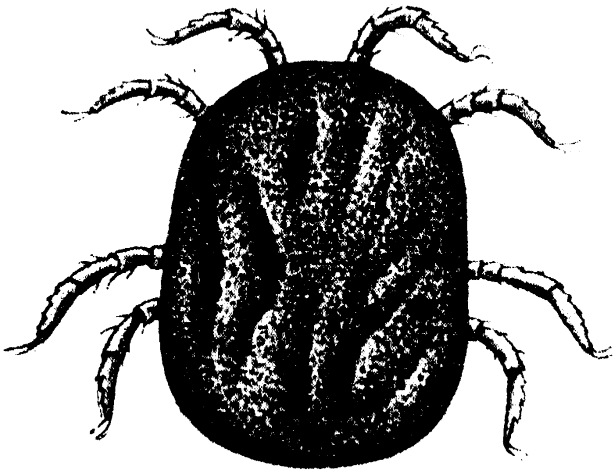

Fig. 59. Tick—dorsal view.

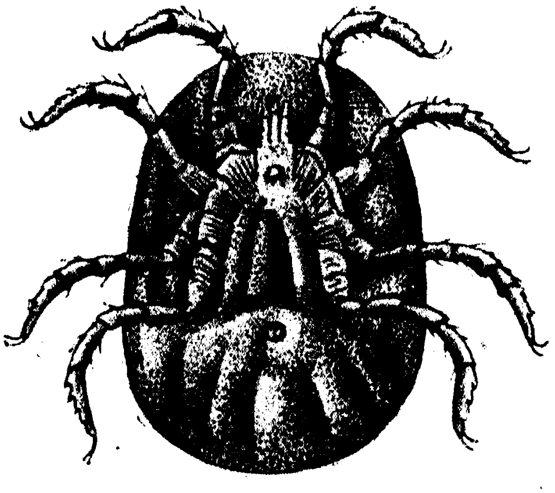

Fig. 60. Tick ventral view.

Yaws differs from syphilis in the following:—

(*a*) Central nervous system never involved. (*b*) No constitutional disturbances. (*c*) Internal organs rarely involved. (*d*) Very readily amenable to treatment with salvarsan. (*e*) Monkeys inoculated with syphilis are immune to yaws, but those inoculated with yaws can be infected with syphilis.

TREPONEMA RECURRENTIS

Source.—All these spirochætes, belonging to the genus treponema are found in blood of the patient, specially during fever, and also in cerebro-spinal fluid, sputum, etc.

Morphology.—Size varies between 12-30μ. Occasionally, very long forms are seen by end-to-end union of two or more organisms. They are actively motile with cork-screw-like lateral oscillatory movement. There are no flagella. Coils are irregular, few and wide open.

Cultivation.—They can be cultivated easily. The medium is composed of one part of rabbit's blood or ascitic fluid and two parts of saline with one-tenth part of 10% peptone broth and put into tubes. Inoculate the tubes with blood of the patient and cover the surface with oil.

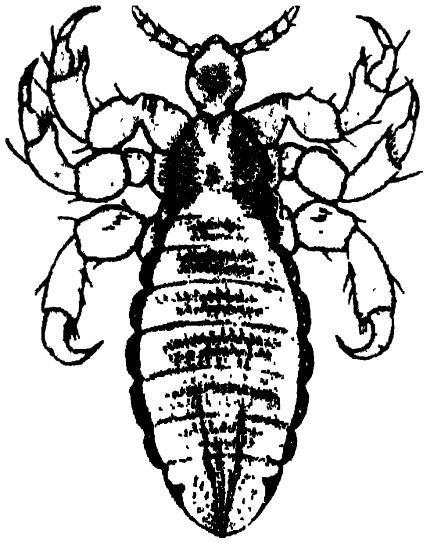

Fig 61. Louse.

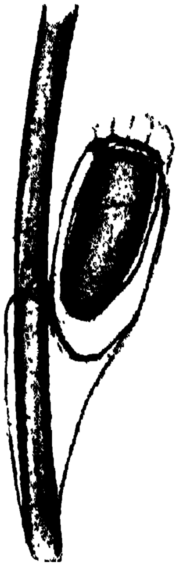

Fig 62. Egg of louse attached to hair. (Nit.)

Pathogenicity.—All these organisms cause relapsing fever. They belong to two groups according to the agents for transmission:—

1. *Tick Relapsing Fever.*—This includes the African and North American varieties, and are conveyed by Ornithodorus moubata.
2. *Louse Relapsing Fever.*—This includes the European, Indian, North African forms, and are generally carried by Pediculus humanus.

Immunity.—Immune serum shows agglutinating, spirochætolysing, precipitins and complement fixation properties.

Inoculation.—Take about 15-20 c.c. of blood from the patient in citrate saline and inject into the peritoneal cavity of white rat or monkey.

S. MORSUS MURIS

(Futaki 1916)

Dobell, Robertson and others no longer consider this to be a spirochæte but a spirillum, *Spirillum minus* (Carter 1887).

Source.—Blood and urine from patients of rat-bite fever.

Morphology.—They are very small forms with thick centre and pointed ends. They possess only two and a half turns.

Pathogenicity.—Causes rat-bite fever, also cat-bite fever.

LEPTOSPIRA ICTEROHÆMORRHAGIÆ
(Inada, Ido, Hoki, Kanako and Ito 1915)

Source.—Urine, and occasionally, blood of patients suffering from Weil's disease or epidemic hæmorrhagic jaundice.

Morphology.—Size 6-12 μ in length. The body of the organism is spirally twisted like a rope with the ends pointed. This spirally twisted organism forms the usual coils like other spirochætes, the pointed ends forming small loops.

Cultivation.—Easy. They grow best in media containing ascitic fluid or serum of laboratory animals.

Pathogenicity.—Causes Weil's disease, generally a disease of the army, and supposed to be transmitted through rat.

LEPTOSPIRA ICTEROIDES
(Noguchi 1918)

Source.—Blood of patients suffering from yellow fever.

Morphology.—Similar to L. icterohæmorrhagiæ.

Cultivation.—Same.

Pathogenicity.—Causes yellow fever which is transmitted by the mosquito, stegomyia.

S VINCENTI
(Vincent 1896)

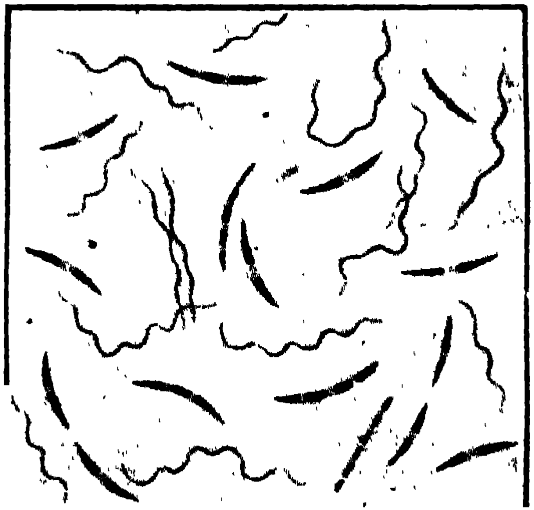

Fig. 63. S. vincenti with fusiform bacillus.

Source :—

1. Throat and other mucous surfaces in Vincent's angina.
2. Naga sore.
3. Ulcus tropicum.

Morphology.—Size 10-20 μ long, slender spirochætes with few open coils. Always associated with fusiform bacilli which show metachromatic granules when stained.

Cultivation.—Must be cultivated along with the fusiform bacillus.

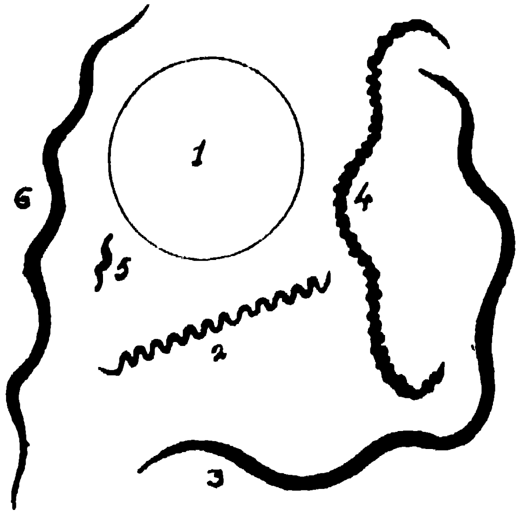

Fig. 64. Schema of different forms of spirochætes, on same scale as human red blood corpuscle.

1. Human red blood corpuscle. 2. Treponema pallidum 3. Treponema recurrentis. 4. Leptospira ictero-hæmorrhagiæ. 5. Spirillum morsus-muris 6 Treponema refringens

Pathogenicity:—

1. Vincent's angina—generally affecting the throat, from which it spreads to the respiratory tract. It is characterised by the formation of a pseudo-membranous patch in the throat, generally mistaken for diphtheria.

2. Naga sore—generally affecting the dorsum of foot, ankle or leg of coolies working in the tea gardens of Assam, especially during rains. It forms an ulcer with offensive odour, and sloughs, and generally affects the deeper tissues.

3. Ulcus tropicum—an affecttion similar to Naga sore.

S. REFRINGENS

Source.— Very common in genital ulcers, and hence commonly identified as T. pallidum.

Morphology.—The organism is larger, coarser, bigger, and with more open coils than T. pallidum.

Staining.—Stains readily with aniline dyes.

Pathogenicity —The organism is non-pathogenic.

CHAPTER XII

VIRUSES AND VIRUS DISEASES

Pasteur, followed by Lœffler, Frosch and several pioneers, established the existence of a distinct class of infective agents which were invisible under the microscope both with stained films as well as under dark-ground illumination. These agents could pass readily through the finest procelain filter candles and the filtrate could initiate diseased processes in sensitive animals. It has also been found that, in some cases, the organisms are ultramicroscopic and occasionally filterable only at one stage. For these reasons these organisms are variously known as the "filterable viruses," "ultramicroscopic organisms," "filter-passing viruses," etc. Evidence has been obtained that at least a hundred or more diseases of animals and plants are caused by these viruses.

That these diseases are caused by so many minute organisms are determined as follows:—

1. Definite period of incubation.

2. Presence of fever and constitutional symptoms.

3. Termination after a fairly uniform course in recovery or death.

4. Characteristic pathological lesions at autopsy.

5. Some of these diseases are transmitted from one man to another by the bite of particular species of infected insects, *e.g.*, dengue by aedes and culex, trench fever by lice, etc.

6. The diseases can be reproduced by subcutaneous or intravenous injection of blood from the patient to a healthy individual. A small quantity of blood from the second individual will be sufficient to infect a third one, from him to the fourth, and so on indefinitely. This shows that the blood contains some *living* substance which can multiply in the human body for several generations.

7. The transmitting agent, *e.g.*, culex and aedes in case of dengue, if allowed to feed on a patient, and subsequently on a healthy individual, produce the disease in the latter.

8. The presence of the virus can be demonstrated by inoculation of cell-free filtrate, extract of tissues or ground-up diseased tissue with successful results.

The Nature of Filterable Viruses.—The first question asked in connection with the infective viruses is whether they are living particulate organisms or not. The important facts which support that they are so, are:—

(1) The capacity of adaptation to changed environments. Thus, when the small pox virus has passed through a sufficient number of calves in succession, it becomes vaccinia and does not return to its original condition with respect to the human being even after repeated passages. Similar facts, in this connection, are the adaptation of herpes to brain tissue, of contagious epithelioma of fowls to pigeons, and of the mosaic disease of tobacco to cucumbers It is difficult to reconcile these facts with the conception of viruses as inanimate substances.

(2) Extreme contagiousness of some of the viruses is out of account with any theory which explains viruses as not living cells but their bye-products.

Regarding the alternative explanation of the nature of viruses, it has been suggested that the virus is a non-living poison elaborated by the affected cells and possessing the power to attack new cells, provoking in them the formation of a further quantity of poison. This view is supported by the following facts:—

1. Difficulty of cultivating viruses in the absence of living tissue. They can be cultivated *in vivo* as in the case of vaccinia virus in testes of rabbits (Noguchi).

2. The difficulties that have been superadded by the discovery of bacteriophages.

Properties of the Viruses.—Most of the viruses are resistant to the action of 50% glycerine. Some of them, notably those of rabies, variola, vaccinia and anterior poliomyelitis, will live in glycerine for weeks without losing their virulence. Like ordinary bacteria, all viruses are destroyed at high temperatures. Some are destroyed at relatively low temperatures (45°C to 55°C) within a short time while others resist a temperature of 70°C. for 15 minutes. Some resist very low temperatures even—2° to—12°C. Some viruses remain active in the dried state for years while some are readily destroyed by weak antiseptics.

Metabolism of the Viruses.—Nothing is known regarding the metabolism of the viruses, as they are invariably present along with the tissue cells.

Serological Reactions.—Like bacteria, the viruses possess antigenic property and produce specific precipitins, agglutinins and complement fixing substances.

Pathogenicity.—All of them are pathogenic although varying greatly in virulence. The virulence is often greatly modified by means of passage, *e.g.*, in case of variola virus.

Diseases take place by direct contagion, generally through nasopharynx, but through skin in case of rabies and through blood in yellow fever, dengue, sandfly fever, etc.

Immunity.—A second attack is generally uncommon. One attack usually protects the individual from subsequent attacks. The immune bodies may or may not be detected in the blood, and it is believed that the reticulo-endothelial system plays some part in the production of immunity.

" Cell Inclusions " and Viruses.—Certain cells of the individual suffering from infection with the viruses exhibit certain atypical bodies or granulations which are known as " cell inclusions " or virus bodies. They occur with such constancy in certain diseases as to be of diagnostic importance. These " inclusion bodies " are either hyaline or granular, basophilic or acidophilic. The following are the most accepted views:—(1) They are accumulations of actual organisms, or special stages of their life cycle, (2) They represent organisms together with cellular reactions due to the infection. (3) They are the products of cell-reaction due to the infection or are the bye-products of cell degeneration. In this connection it is to be noted that Goodpasture and Woodruff have described a method of isolating single virus bodies of fowl pox after tryptic digestion of the infected epidermal cells. When this single inclusion body is washed with saline and inoculated into the skin, a typical fowl-pox lesion containing the characteristic cell inclusions is reproduced.

DISEASES CAUSED BY THE FILTERABLE VIRUSES

Variola and Vaccinia.—Although there is a great deal of controversy between the relationship of these diseases, it is generally regarded that vaccinia is a modified form of variola. As no specific organism could be distinctly connected with these diseases, they are regarded as being caused by filterable viruses. As mentioned before, they can live in glycerine without loss of virulence.

Acute Anterior Poliomyelitis (Infantile Paralysis).—The virus causing this disease is filterable. It generally affects children, either sporadically or epidemically, through the naso-pharyngeal mucous membrane and is eliminated by the same and is transmitted to other children through "carriers" who themselves remain unaffected. It may also be carried by various insects, of which house fly, bed bug, etc , are the common suspects. Mosquitoes and fleas are probably not the vectors. The virus can retain its virulence for a considerable period in glycerine, sterile milk, sterile water and even in the dust of the patient's bed-room. It is destroyed at 45°C-50°C.

Rabies (Hydrophobia).—The virus is ultramicroscopic and is characterised by the formation of Negri Bodies, which are round or oval, varying in size from small structureless bodies to bigger ones and generally found in the cells of the central nervous system. They present a circle of chromatoid granules round a central granule. Their constant presence is of great diagnostic importance. The infection in man and animals takes place through bites from rabid dogs, cats, jackals or other animals. There is an incubation period which varies from 3 to 12 weeks, generally 10 weeks for man, although it may prolong to 2 years. Pasteur's method of prevention and treatment is the greatest boon to humanity. For details of Pasteur's method, *see* chapter on *Immunity*.

Dengue.—This is also caused by a filterable virus and is transmitted by the mosquito, Aedes ægypti and perhaps by Culex fatigans as well.

Yellow Fever.—The virus is filterable. Sanarelli's B. icteroides is now regarded as a casual invader and not the causal factor. The mosquito Stegomyia fasciata, now called Aedes argenteus, is the transmitting agent.

Phlebotomus (Sandfly) Fever.—This is caused by a filterable virus and is transmitted by the sandfly Phlebotomus papatasii.

Measles.—The blood and the oral and nasal secretions contain the filterable infective virus. The virus is present 24 hours preceding to 24 hours following the appearance of the eruptions.

Trench Fever.—The causative filterable virus is found in the blood and urine of the patient. It is transmitted by the louse.

The other Diseases of Man and Animals caused and supposed to be caused by Filterable Viruses are trachoma, herpes, alastrin, pleuro-pneumonia in cattle, swine fever, rinderpest (cattle plague), foot and mouth disease, canine distemper, sheep pox, mosaic disease of the tobacco plant, potato mosaic, etc.

Diseases of Questionable or Doubtful Origin.—Scarlet fever, malignant diseases. typhus fever, mumps, pellagra, rheumatic fever, encephalitis lethargica, cholera nostras, summer diarrhœa of infants, beriberi, epidemic dropsy, etc.

CONSIDERATION OF SOME TECHNICAL POINTS

The smallest size of a particulate body visible under the microscope is 0 1 to 0 2 μ, while with an ultramicroscope, the smallest size visible is 1 μμ to 0.1 μ. (1 μ is equal to 1000 μμ). This is generally seen in case of colloidal bodies which are thus called *submicrons*. The molecules in true solution are smaller than 5 μμ, and are generally known as *amicron*. The visibility of a particulate body depends on its capability of being illuminated and mainly on the refractive index of the suspending medium which must be different from that of the body to be rendered visible.

The points leading to technical difficulties for the determination as to whether a virus can pass through the filter depends on the following factors:—(1) Type of the porcelain filter. Berkefeld V-filter is not suitable, as the pores are fairly big, allowing bigger particles to pass through. It is besides very fragile and can be cleaned only with difficulty. (2) The relative size of the filter and the particulate body (3) The concentration, viscocity and the nature of the protein content of the suspending material. (4) Time allowed to filter. (5) Temperature, pressure and hydrogen ion concentration of the medium.

CHAPTER XIII

CHARACTERS USED FOR CONFIRMATION OF THE MOST IMPORTANT PATHOGENIC BACTERIA

(Stain Smears with Methylene Blue and by Gram's Method)

If the Organism is a Coccus and Gram Positive

Staphylococcus.

MORPHOLOGY.—Arranged in clumps or bunches.—AGAR.—Moist, shining growth and note colour of the colonies, whether aureus, albus or citreus.

Streptococcus.

MORPHOLOGY.—Arranged in chains. AGAR or BLOOD AGAR.—Minute, discrete, colourless colonies

Pneumococcus.

MORPHOLOGY.—Arranged in pairs Lanceolate shape. Presence of a distinct capsule, which is lost in culture and reappears when examined from the inoculated animal. BLOOD AGAR.—Small transparent colonies. *Differentiation from Streptococcus.* Pneumococcus is soluble in bile and ferments inulin.

Inoculate mice and recover the organism from heart's blood.

If the Organism is a Coccus and is Gram Negative.

Gonococcus.

MORPHOLOGY.—Arranged in pairs Each coccus kidney shaped. Usually present inside pus cells, may be outside, in urethral discharge. CULTURE.—Agar—no growth. Blood Agar or media containing hydrocele fluid or other tissue-fluid—small, discrete, colourless colonies. Serum-broth.—Faint growth with no turbidity.

Meningococcus.

MORPHOLOGY.—Arranged in pairs. Usually present inside pus cells, in cerebro-spinal fluid. CULTURE.—Agar—fair growth. Nasgar or trypagar medium—more luxuriant growth. Serum broth—Fair growth with turbidity.

If It is a Bacillus and Gram Positive

B Tuberculosis.

MORPHOLOGY.—Arranged singly or in pairs in the form of "V." STAINING.—Acid-fast. CULTURE.—Agar and broth—no growth. Glycerine agar or broth—growth specially in subcultures; growths are of dry, dull, wrinkled appearance. Egg medium (Dorset's)—grows. INOCULATION.—Inoculate guinea-pig—animal shows lesions in three weeks

B. Lepræ.

MORPHOLOGY.—Arranged in bundles and are grouped together. STAINING.—Acid-fast. CULTURE.—Not satisfactory in any medium. INOCULATION.—Not satisfactory in any animal.

B. Anthracis.

MORPHOLOGY.—Large bacillus with square-cut ends. Central spore—best seen when stained. STAINING.—Special method for spore. CULTURE.—Agar.—Abundant growth with characteristic wavy (the so-called "wooly") margin. INOCULATION.—Inoculate guinea-pig subcutaneously—bacilli found in heart's blood, spleen and other organs.

B. Tetani.

MORPHOLOGY.—Bacillus with large terminal spore—the so-called drum stick appearance. STAINING.—Special method for spores and flagella. CULTURE.—Grows only in anærobic condition. Deep stab culture—growth shows fine lateral threads.

B. Ærogenes Capsulatus.

MORPHOLOGY.—Bacillus with subterminal spore. Presence of capsule STAINING.—Special method for spore and capsule. CULTURE.—Grows only in anærobic conditions. Cooked meat medium shows formation of gas and pink-colour in the medium. INOCULATION.—Inject the culture into a rabbit intravenously, kill the animal and incubate the dead animal at 37°C. for six hours. The animal will swell up with formation of gas in all tissues. Isolate the organism in pure culture from the liver or heart.

Vibrion Septique.

MORPHOLOGY.—Bacillus with subterminal spores, motile. CULTURE.—Cultivate anærobically.

B. Diphtheria.

Stain with Neisser's stain. MORPHOLOGY & STAINING.—Irregular shape of the bacilli, well-marked granules—the so-called metachromatic granules, club-shaped involution forms especially in old culture. The granules are stained blue and the body of the organisms brown. CULTURE.—Inoculate.—(*a*) Loeffler's blood serum medium. (*b*) Egg medium—Ordinary or tellurite egg medium.

If it is a Bacillus and Gram Negative

B. Influenza.

CULTURE.—Cultivate on blood medium Colonies look like minute drops of dew.

B. Pestis.

MORPHOLOGY—Short, oval bacillus showing bipolar staining. CULTURE.—Ghee broth—shows stalactite growth. Salt agar—shows involution forms. Agar—grows feebly. Blood medium—luxuriant growth. INOCULATION.—Inoculate rat or guinea-pig—death in 1-3 days, recover the organisms from spleen, blood or gland.

B. Coli.

MORPHOLOGY.—Motile. CULTURE—MacConkey's medium—red colonies. Ferments all sugars with production of both acid and gas. Satisfies *Flaginac* test. AGGLUTINATION.—Agglutinated by specific antiserum.

B. Typhosus.

MORPHOLOGY.—Motile. CULTURE.—MacConkey's medium—colourless colonies. Ferments glucose with production of acid only and no gas, does not ferment lactose. Potato medium—invisible growth. AGGLUTINATION.—Agglutinated by anti-typhoid serum.

B. Shiga.

MORPHOLOGY —Non-motile. CULTURE.—MacConkey's medium—colourless colonies Ferments glucose with production of acid only and no gas, does not fement lactose and mannite. AGGLUTINATION —Agglutinated by anti-Shiga serum.

B. Flexner.

MORPHOLOGY.—Non-motile. CULTURE.—Same as *B. shiga* except that it ferments mannite. AGGLUTINATION.—Agglutinated by anti-Flexner serum.

B. Paratyphoid *A. and B.*

MORPHOLOGY.—Motile. CULTURE —MacConkey's medium—colourless colonies Ferments glucose with production of both acid and gas, does not ferment lactose.

AGGLUTINATION.—Agglutinated by anti-paratyphoid serum A. and B. respectively.

B. Gærtner.

MORPHOLOGY.—Motile. CULTURE.—McConkey's medium—colourless colonies. Ferments glucose with production of both acid and gas, does not ferment lactose. AGGLUTINATION.—Agglutinated by the anti-Gærtner serum.

B. Pyocyaneus.

MORPHOLOGY.—Motile CULTURE.—Agar, broth or other medium—growth with production of green colour throughout the medium. Shake up culture with chloroform—the chloroform is coloured green.

V Choleræ.

MORPHOLOGY.—Curved, comma-shaped spirillum, often forming "S" or spiral forms by joining two or more organisms. Motile, often assuming "fish-stream" appearance. CULTURE.—Peptone water—organisms grow very rapidly forming a pellicle on the surface. Add few drops of sulphuric acid (conc.)—Cholera red reaction (presence of both indol and nitrites.). Dieudonne's medium.—Only cholera vibrio will grow and none else. Taurocholate agar medium —Very suitable. AGGLUTINATION REACTION.—Agglutinated by anti-cholera serum PFEIFFER'S TEST.—Applied to guinea-pig is positive.

Spirochoeta Pallida.

MORPHOLOGY.—Spiral-shaped organisms with six to eight regular, uniform curves and showing characteristic movements when examined by the dark ground illumination. Other methods are:—Indian ink method. Congo red method. STAINING —Fontana's method. Leishman's method. Giemsa stain CULTURE.—Noguchi's method. INOCULATION.—Inoculate the testis of a rabbit.

		CULTURE.		
		Broth.	Special media.	Liq. gelatin.
Cocci	All exo St c g St t r pn t c	Same as agar. **strepto-** not turbid rest-turbid.	**Gonococcus**-media containing uncoagulated body fluid, *e.g.*, blood, hydrocele fluid, **meningococcus**-nasgar, trypagar media.	Gelatin culture not suitable in this country.
Baci	All ex aci an hæ- lic ps. Gr cre col an op Ant sho wo na	Same as agar. All produce turbidity.	**Tubercle B.**—Glycerinated media, inspissated blood serum medium, egg or Petroff's media. **Lepra B.**—cannot be cultivated. **Tetanus—& gas gangrene—** anærobic methods. **Hæmophilic group**-media containing blood. **B. pestis**-Ghee broth, salt agar. **Intestinal group**— McConkey, Conradi-Driglaski, Endo media and those containing sugar and indicator. (for sugar reaction *see* text). **Diphtheria B.** Lœffler's blood serum or egg medium.	
Sp	ho ro well	Same as agar.	**Cholera**—Durham's peptone water, Dieudonne medium, taurocholate agar.	
	iræ- s- not.		**Spirochætes**—Noguchi's anærobic methods.	

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CHAPTER XIV

PROTOZOA

Protozoa (*protos*—primitive, and *zoon*—living animal) are unicellular animal organisms. Dobell defines them as non-cellular animals, as some groups of protozoa, *e.g.,* the amœbæ, possess no cell-wall, the protoplasm remaining naked.

Like bacteria, protozoa are present almost in every place. Practically, no animal is free from the invasion of the protozoa.

The protozoa may be free-living, harmless commensals, or harmful parasites.

Classification of the Protozoa:—

Phylum	Protozoa			
Subphylum	Plasmodroma			Ciliophora
Class.	Rhizopoda (Sarcodina) or Amœba	Mastigophora or Flagellata	Sporozoa or Spore-forming Protozoa	Ciliata or Infusoria

Morphology.—A protozoon consists of a single cell composed of protoplasm. The protoplasm is differentiated into the nucleoplasm and the cytoplasm.

The nucleus consists of a nuclear membrane enclosing one or more karyosomes or nucleoli. Within the nuclear membrane there is a reticular structure, called the nuclear reticulum, the interstices of which is filled up by a fluid structure, the nuclear sap. The karyosomes and numerous other fine granules are composed of chromatin which stains deep red with Leishman stain and black with iron hæmatoxylin.

The following terms are used to denote the different structures of the protozoa:—

Karyosome.—Nucleoli.

Centrosome.—A fine granule inside the nucleus which regulates cell division.

Blepharoplast.—Centrosome regulating movement of the protozoa.

Amitosis.—Direct division of the cell, the nucleus divides into two, followed by division of the cytoplasm, and then, of the nuclear membrane.

Mitosis.—Indirect cell division or karyokinesis.

Cytoplasm may be clear, or may contain food granules, vacuoles, which may be contractile, either containing food, water or excretions, foreign bodies, *e.g.*, bacteria, and food reserve, *e.g.*, glycogen, etc.

SARCODINA

(*Sarcos-flesh*)

They have got no cell membrane, are constantly moving by means of projection of pseudopodia, and multiply by fission or cyst formation.

Distribution.—Universal, occurring as free-living, harmless commensals, or as harmful parasites.

Free-living—in pond water, other drinking water, infusions, etc. *Commonest species*—A. limax. They are rarely present in human fæces. They have a big central or eccentric vesicular nucleus with contractile vacuoles They may appear in human fæces from the dirty water used for washing the bed pan. These forms are termed by Dobell as "Coprozoic" amœbæ. They can be cultivated readily in Musgrave and Clegge's medium.

Parasitic amœbæ are called *entamœbæ.* They are wide-spread. Man as well as the lower animals are parasitised by them. Some of these in lower animals are E. blattæ of cockroach, E. ranarum of frog, E. muris of rat, etc.

Human entamœbæ are:—

1. E. histolytica,	4. Iodamœbæ butschlii,
2 E. coli,	5. Dientamœbæ fragilis,
3. Endolimax nana,	6. E. gingivalis.

The peculiarity of the parasitic amœbæ is that they live only in one host and in that host alone; as, for example, E. histolytica, the human parasite, will not infect frog, nor E. ranarum of frog infect man.

ENTAMŒBA HISTOLYTICA

Discovered in Russia by Lœsch in 1875.

This is the only patnogenic amœbæ of the human intestines. It is called *histolytica* (*histos*—tissue, *lysis*—destruction) on account of its habit of dissolving the tissue by a proteolytic enzyme and feeding on the liquefied material. It lives in the mucous membrane of the large intestine, where it multiplies and becomes encysted. It is found in the conditions and the states mentioned below:—

(*a*) Acute amœbic dysentery—motile vegetative stage.
(*b*) Convalescence after amœbic dysentery—precystic stage.
(*c*) Amœbic carriers (either contact or convalescent)—cystic stage.

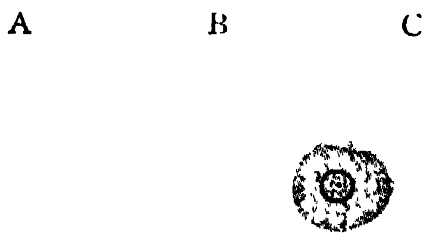

Fig. 65. Entamœba histolytica × 500.
A. Vegetative form. B. Precystic form. C. Cystic form.

Morphology.—Size varies a good deal between 20-30 μ .

Motility.—They are actively motile. The movement is maintained by *pseudopodia* which are long, fine, finger-like projections and consist only of ectoplasm.

Cytoplasm.—Consists of two elements, which are:—(*a*) Ectoplasm—distinct, clear and forms about one-third of the cytoplasm. (*b*) Endoplasm—finely granular, with or without red blood cells, never contains bacteria or other food particles, hence they are sterile, and their presence in liver abscess does not produce secondary contamination. Dying entamœbæ show the presence of bacteria, vacuoles, yeast, etc. These are not the substances ingested by the amœbæ, but are, in their turn, parasitised by them. The presence of these degenerative pictures led the workers, working with stale fæces, to describe new species with mistaken life cycles and subsequent confusion in the literature.

Nucleus.—Indistinct in the living condition, but becomes clear when stained. It consists of a nuclear membrane, with a large number of finely granular chromatin on its inner aspect, which gives it a beaded appearance. The karyosome is very fine and placed centrally. This is known as the " histolytica type of nucleus."

Multiplication.—By binary fission, or by *Cyst formation,* which is preceded by a condition known as the pre-cystic stage in which it assumes a very small size (5-15 μ -E. minuta), all pseudopodia are withdrawn, all red cells are thrown out, endoplasm becomes clear and secretes the cyst wall. The nucleus divides into two, and ultimately into four. A form of reserve substance is produced, and remains in the cyst as refractile bars, called chromatoid bars. They stain black with iron hæmatoxylin, but are not well stained with iodine. Cysts are formed in the lumen of the gut and are passed out with formed fæces. They are very resistant. The mature cysts, eaten up along with the contaminated food, pass intact through the stomach into the small intestine where they hatch and liberate four amœbæ. These then pass into the large intestine where they enter into the mucous membrane and live in the manner detailed above.

Cultivation.—They can be cultivated on Locke-egg-serum medium The best method is to inoculate a kitten per rectum with the fæces containing motile amœbæ or cysts. About 70% of inoculated animals die of acute amœbic dysentery during a week. Bœck and Drobohlav in 1924 successfully cultivated E. histolytica, and since then various workers have been able to repeat and corroborate their method. Bœck and Drobohlav's medium consists of a liquid medium standing on a solid one. The liquid portion is inoculated with mucus. E. histolytica grows in two to three days but is destroyed by the overgrowth of bacteria after that period. For that reason subculture must be made every two days. Perfectly fresh material from the kitten may be cultivated into the above culture medium.

Pathogenicity.—Ent. histolytica lives in the mucous membrane of the large intestine,* where they secrete a powerful cytolytic ferment which liquefies the tissue and feed on the liquefied material. This produces an ulcer of the intestine. The ulcer is caused not by the mechanical loss of the tissue produced by the passage of the amœbæ through the wall but by the lytic effect on the tissue itself. This is also corroborated histologically by the fact that the amœbæ are nearly always found surrounded by an area of liquefied material. Nature is also busy in the reparative process. When the process is very mild, probably there will be no inconvenience to the host. In some instances, it may lead to occasional diarrhœa lasting for a short time. Only when they become very active, they produce dysenteric lesions, often leading to gangrenous changes and fatal termination. More or less extensive ulceration of the colon caused by E. histolytica may remain without any symptom. A characteristic periodic variation in intensity of the intestinal lesion is often seen. During the course of amœbic dysentery, a large number of actively motile amœbæ are passed out with the evacuations. Occasionally, they migrate through the portal vein and reach the liver, where, again, owing to their histolytic nature, they cause necrosis of liver cells with the formation of abscess of the liver. As there is no septic organism in such an abscess, the contents remain sterile, unless secondary contamination, specially with B. coli, takes place. Similar amœbic abscesses of the spleen, brain and other parts are also on record The organisms remain in the wall of the abscess cavity and not in the pus. In carriers, liver abscess may occur without dysenteric symptoms.

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* Recently Boyers, Kofoid, and Swezy demonstrated the presence of the vegetative form as well as the cysts of all stages in the duodenum.

It is a peculiar feature that the amœbæ can scarcely live for more than 2 or 3 hours outside the human colon except in the cystic form. Those that migrate to the liver and other parts cannot encyst. So, in acute dysentery or liver abscess, large numbers of the organisms are destined to be destroyed. Thus, it may be said that amœbiasis is, strictly speaking, a disease of the amœbæ and not of man, and, in case of death of the host, all the E. histolytica will die.

Infectivity.—A man passing the actively motile amœbæ during an attack of acute amœbic dysentery is absolutely harmless to others, as the organisms die out rapidly before being encysted.

Men just convalescent from amœbic dysentery, or having been in contact with such a case passing large number of cysts with more or less formed fæces, are very dangerous to others. They are known as carriers and, as mentioned above, are of two kinds, namely, (*a*) convalescent carriers, who have suffered from dysentery and have recovered and (*b*) contact carriers who have never suffered from active dysentery but have been infected during contact with a patient.

ENTAMŒBA COLI

Discovered in Calcutta by Lewis in 1870 in the evacuations of a cholera case.

E. coli is a harmless commensal. It lives in the lumen (not in the mucous membrane) of the large intestine. It feeds on the human excreta, and, so, may be called a scavenger.

Morphology.—SIZE.—This is the largest entamœbæ of man, measuring from 20-40 μ.

Motility.—Sluggish.

Pseudopodia.—Big, rounded, consists of endoplasm only and practically no ectoplasm (*cf.* E. histolytica).

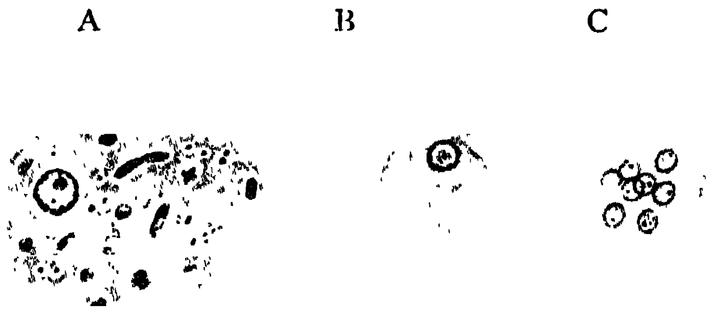

Fig. 66. Entamœba coli ×500.
A. Vegetative form. B. Precystic form. C. Cystic form.

Cytoplasm—Consists of (*a*) Ectoplasm—almost absent, and (*b*) Endoplasm—densely granular and loaded with food vacuoles, cysts of intestinal protozoa, various forms of food-stuffs, bacteria, yeast, etc., *but never red blood cells.*

Nucleus.—Very prominent, especially when stained, and clearly visible in living condition. It consists of a sharp nuclear membrane with a large number of granular chromatin placed on its inner aspect and enclosing a *big eccentrically placed karyosome.* This is known as the "coli type" of nucleus.

Multiplication.—By binary fission, or by *Cyst formation.* Like E. histolytica, this is preceded by the pre-cystic stage. At this stage it becomes smaller, and the pseudopodia are withdrawn. The endoplasm becomes clear. All food particles, vacuoles and debris disappear. Finally, movement stops altogether and is followed by the secretion of the cyst wall. The nucleus divides into 2, 4, and subsequently into 8 nuclei. The cyst is larger than the pre-cystic stage and measures 15-20 μ. Dobell has seen further division of the nucleus, with formation of a very big cyst (giant cyst) containing even 22 nuclei. Glycogen is abundant in the 2-nucleate stage.

Chromatoid bodies which are fairly constant in E. histolytica cysts are very rare in the E. coli cyst.

Occurrence.—Like E. histolytica (vegetative forms) the E. coli are also found in persons when the fæces are of liquid consistency with some pre-cystic forms also and with no cysts. Cysts are found only when the fæces is formed.

Mode of Infection.—Experiments on prisoners by Walker and Sellards showed that when fresh fæces containing E. coli cysts are ingested in gelatine capsules, they commence to pass E. coli cysts with their fæces in an average period of 4 to 7 days.

Pathogenicity.—They are found nowhere but in man. They are non-pathogenic.

Differentiation between E. histolytica and E. coli.

	E. histolytica	E. coli
Size	20-30μ	20-40μ
Motility	Very active	Sluggish.
Pseudopodia	Thin, finger-like, consist of ectoplasm only.	Blunt, rounded, consist mostly of endoplasm.
Ectoplasm	Prominent	Scarcely seen.
Endoplasm	Finely granular with presence of only R.B.C.	Densely granular, loaded with bacteria, yeast, vacuoles, etc., but never R.B.C.
Nucleus (stained)	Ring like type with central karyosome.	Ring-like type with eccentric karyosome.
Cyst	Four-nucleated with the presence of chromatoid bodies. Size 6-18 μ.	Eight-nucleated with no chromatoid bodies. Size 15-20 μ.

ENDOLIMAX NANA

These inhabit the lumen of the small intestine, and are harmless commensals.

Morphology.—They measure 6-12 μ (one red blood cell measures 7μ), are sluggishly motile; pseudopodia are small and blunt; ectoplasm scanty; endoplasm loaded with food particles, bacteria, yeast, vacuoles, etc. Never ingest red cells. Nucleus consists of a big mass of eccentrically placed karyosome.

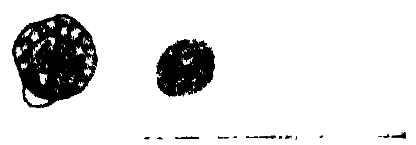

Fig. 64. Other species of amœbæ.

Cyst Formation.—This is preceded by a pre-cystic stage.

Adult cyst contains 4 nuclei. It is of the size of a red blood cell.

Pathogenicity.—It is non-pathogenic.

IODAMŒBA BUTSCHLII

Cysts were discovered by Wenyon and O'Connor in 1915 and adults by Dobell in 1917. They are harmless commensals.

Morphology.—Size 15-20 μ, sluggishly motile, pseudopodia blunt, ectoplasm scanty, endoplasm loaded with food particles, vacuoles, etc., but never red blood cells. Nucleus consists of a big central karyosome.

Cyst Formation.—It is preceded by a pre-cystic stage. All food particles are thrown out, and a thick cyst wall is secreted with the formation of glycogen vacuoles. There is a single nucleus. The cytoplasm contains one or two big vacuoles filled with glycogen, which serves as reserve material and hence called "iodine-cysts."

Staining Peculiarity.—Glycogen is soluble in water, hence any watery stain will show big empty vacuoles. When stained with an alcoholic solution of iodine, the cyst becomes very prominent, staining a deep mahogany-brown.

DIENTAMŒBA FRAGILIS

This is also a harmless commensal. It is rarely found, and, as the name implies, it contains two nuclei in the vegetative stage, and is very fragile. Its cyst is not known.

ENTAMŒBA GINGIVALIS

It has been supposed to cause pyorrhœa alveolaris. It lives in the tartar on teeth and pus of the pyorrhœic pockets.

Morphology.—Similar to E. coli, only smaller in size—10-20 μ They never ingest red blood cells.

CHAPTER XV

MASTIGOPHORA

Flagellata (*Mastix=a whip*)

The flagellates occur widely in nature, just like the bacteria and amœbæ. They exist as free-living, harmless comensals, as well as dangerously harmful parasites.

They are called flagellates, because they possess one or more whip-like processes, called the flagella. A flagellum is a long whip-like mass of protoplasm, which is mainly used for the purpose of locomotion of the parasite. The flagellum arises from a so-called "basal granule," which takes the chromatin stain and is probably derived from the nucleus, and the movement is regulated by a chromatin particle, variously called kinetonucleus or para-basal body. The word blepharoplast is used by various writers to denote different structures (basal granule, parabasal body, micronucleus, centrosome, etc). Hence it is safer to avoid this term. The flagella may be single or multiple.

Classification.—The scientific classification of mastigophora is too cumbersome. It is more convenient to classify them under two headings:—

(*a*) Intestinal flagellates—inhabiting the human intestines.
(*b*) Hæmoflagellates—inhabiting the human blood.

INTESTINAL FLAGELLATES

These inhabit the intestine of man, and are found in the fæces through which they leave the host. They are usually non-pathogenic, except the Giardia. They are as follows:—1. Giardia (lamblia) intestinalis. 2. Trichomonas. 3. Chilomastix (tetramitus) mesnilli. 4. Enteromonas. 5. Embadomonas. 6. Coprozoic (*e.g.*, Cercomonas, Bodo, Prowazekia, etc.)

I. GIARDIA (LAMBLIA) INTESTINALIS

This is the only pathogenic organism of this group of flagellates. They usually remain in the intestines as perfectly harmless, but may cause diarrhœa. They live in the small intestine, and are often found fixed to the mucous membrane. They die as soon as they come out of the host, and so the movement is scarcely seen during examination of the fæces, specially, in specimens passed some time back.

Morphology.—The organism is flattened and bilaterally symmetrical. It is pear-shaped. Size—The length of the body from its rounded anterior end to the tip of the caudal process is about 10-8 . The posterior end is pointed, the anterior end flat and broad. It possesses a deep *kidney-shaped depression* on the anterior end which is used in fixing on the mucous membrane. In this depression, there are two big oval anteriorly placed *Nuclei*, each containing a cluster of karyosomes placed in the centre, which look like the pupils of the eyes. There are two slender rods running nearly the whole length of the parasite which serve the purpose of the skeleton, and are known as the *axostyle*. There are 8 *Flagella* in 4 pairs as follows:—

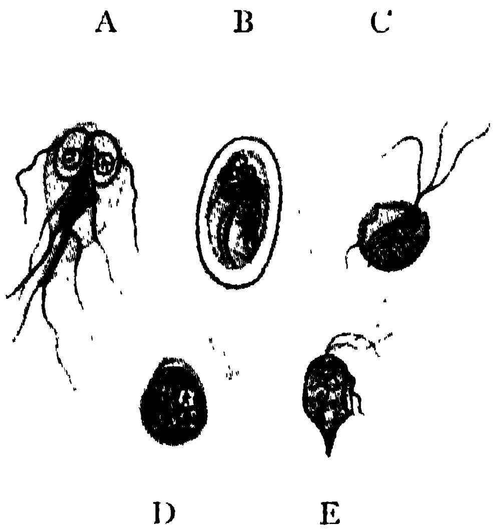

Fig. 65. A. Giardia intestinalis (vegetative form).
B. Giardia intestinalis (cystic form).
C. Trichomonas
D. Chilomastix (cystic form).
E. Chilomastix (vegetative form).

(*a*) One pair—the anterior one—arises from the anterior end of the axostyle, and the two cross each other and pass round the anteriolateral margin of the cup-shaped depression, until they become free from the sides.

(*b*) The middle pair originates from the anterior end of the axostyle, proceeds backwards to the posterior margin of the depression, and ultimately, emerges as free lateral flagella.

(*c*) A ventral pair, thickest of all, originates from the posterior edge of the depression as free flagella.

(*d*) The last pair originates from the posterior end of the axostyle as free flagella.

In the centre, there is a mass of substance known as the *Parabasal Body*.

Multiplication takes place by longitudinal binary fission.

Cyst Formation.—This takes place in the lower part of the intestines. The cysts are usually very small in size 7—10 μ. The cysts have clear wall enclosing the parasite which is shrunken away from the cyst wall. The parasite shows the presence of two nuclei and rudiments of the third pair of flagella. This is divided into two by an oblique line of demarcation inside the cyst. The cyst develops into two parasites when it gets entrance into a new host. The cysts are present in excessive numbers, but the peculiarity of the cysts is that they may be found one day, whereas a subsequent examination may give a negative finding, only to reappear in large numbers on a subsequent occasion.

Cultivation.—Unknown.

Pathogenicity.—Usually harmless, but may produce diarrhœa or dysentery, which is usually fatal.

2. TRICHOMONAS..

It is a common parasite inhabiting the human alimentary canal, specially the cæcum and in acid vaginal secretion.

Morphology.—The cell is very delicate and is lined by a thin membrane called periplast. It measures 10-15 μ in length. It is actively motile. It is more or less rounded with a pointed tail end. The *Nucleus,* placed anteriorly, contains a big central karyosome and a few chromatin granules. There are a few basal granules in front of the nucleus, from which a group of flagella arises. A dying trichomonas shows slow movement of the undulating membrane, which often appears as a pseudopodia. This led Castellani to classify this form—wrongly—as a new amœba, the E. undulans. There are four free *Flagella* (3?) originating anteriorly, and another big one, originating from the same situation which passes backwards, and forms the *Undulating Membrane.* This is projected further back, to form the free posterior flagellum. This is the only intestinal protozoa of man provided with an undulating membrane which moves continuously. (*See* Fig. 65).

One *Axostyle* or spinal column originates from the chromatin granules (basal granules), and serves the purpose of stiffening; the anterior end contains a slit which is called the mouth or *Cytostome.*

They often ingest red blood corpuscles.

Cultivation.—They grow luxuriantly in Row's hæmoglobinized serum medium in about three days.

Multiplication.—By binary fission.

Cyst Formation.—Unknown.

Varieties—(1) In some cases there are five free anterior flagella which were first described by Chatterjee and termed *Pentatrichomonas.* This is the only species prevalent in Calcutta. (2) *T. vaginalis*—found in cases of leucorrhœa with acid discharge. *Pathogenicity.*—They are non-pathogenic but are very often found in dysenteric stools caused by Shiga and Flexner bacilli.

3. CHILOMASTIX

(*Tetramitus mesnilii*)

They inhabit the upper part of the colon.

Morphology.—Resemble trichomonas, but are not so delicate. They possess (*i*) three free anterior flagella; (*ii*) fourth flagellum is present in the mouth and helps to capture food, (*iii*) one nucleus; (*iv*) one big mouth (cytostome), hence also termed *Macrostoma;* (*v*) no undulating membrane.

Multiplication.—By binary fission.

Cyst Formation.—Takes place in the lumen of the gut, and cysts pass out with formed fæces. They are about the size of a red corpuscle. They contain the outline of the parasite, a portion of the cytostome, the nucleus and the 4th flagellum. (*See* Fig.) They occur in very considerable numbers when present.

Pathogenicity.—It is non-pathogenic.

4. ENTEROMONAS

These are very small. They contain (*i*) a big nucleus; (*ii*) four flagella, all originating from one point, three anterior and the fourth one proceeds backwards to form the tail.

Multiplication.—By binary fission and cyst formation.

5. EMBADOMONAS

More or less resemble enteromonas.

6. COPROZOIC FLAGELLATES

They are always found in stale fæces, and are accidental inhabitants of the fæces. They are either deposited in the passed fæces from the water used for the bed-pan, or the cysts pass through the human intestine as such, and then grow in the passed fæces.

They are of three varieties—Cercomonas, Bodo and Prowazekia.

HÆMOFLAGELLATES

Most of the hæmoflagellates (so called because they usually inhabit the blood) are grouped under the Order Binucleata,* as they possess two nuclei. They are of great importance in human pathology, as some of them cause deadly disease in man. They are also found in lower animals, and even plants are not spared. They may be free-living, harmless commensals or harmful parasites.

Morphology.—They are more or less elongated organisms with two nuclei, (*i*) micronucleus—small in size, stains deeply and regulates movement, hence also called *kinetonucleus;* (*ii*) macronucleus—bigger in size, stains less deeply and regulates nutrition of the cell, hence also called *trophonucleus.* They have one flagellum (trypanoplasma contains two), with or without forming undulating membrane.

Classification :—Order Binucleata.*

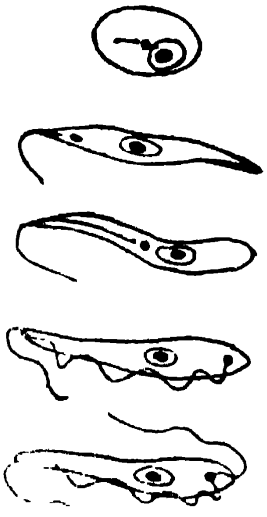

Fig. 66. Hæmoflagellates

Genus.

Leishmania, same as Herpetomonas. Exists in 2 forms: (*a*) in tissue, rounded, no *F*; *N* and n side by side, and (*b*) in culture herpetomonas-like.

Herpetomonas, *N* central, *n* anterior, *F* single, ant

Crithidia, *N* central, *n* just in front of *N*, *F* at ant. end, originates from *n* by axostyle (trace of undulating membrane).

Trypanosoma, *N* central, *n* post, *F* ant. originates from *n* and passes forwards along the body as undulating membrane.

Trypanoplasma (also called cryptobia), *N* central, *n* ant., 2 *F* (one ant. and the other posterior, both from *n*), posterior connected with *n* by undulating membrane

N.B.—*N* macronucleus, post. Posterior, *n* micronucleus, *F* flagella. Ant. Anterior.

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* Modern workers do not accept the term "Binucleata," as they do not regard as valid the "binuclearity hypothesis" of Hartmann, which runs as follows:—(*a*) That the micronucleus consists of nuclear chromatin.

(*b*) That the micronucleus originates from macronucleus.

(*c*) That it divides by mitosis.

The modern opinion is to classify the macronucleus as the uncleus of the cell and the micronucleus as the "parabasal body" or simply "parabasal."

HERPETOMONAS

Herpetomonas usually inhabit the intestines of insects, and hence their recognition is important in the study of the transmission problem of *kala-azar.*

They are normally found in abundance in the gut of house fly (Herpetomonas muscæ-domestica), dog flea (H. cteno-cephali), culex, hemiptera-bugs, etc.

Morphology.—See classification above.

LEISHMANIA

These are the organisms causing *kala-azar, oriental sore* and *espundium.*

History of Discovery.—In 1903 Leishman discovered some peculiar organisms from the spleen pulp of a soldier who had died of Kala-azar. He attributed these bodies as degenerated forms of trypanosoma. Shortly after, Donovan observed the same parasites from spleen puncture of living patients in Madras. Subsequently, Wright again observed similar organisms in the smear from Delhi boil. At the beginning, there was much controversy as regards the nature of the organisms and their proper position amongst the protozoa.

All these organisms are now known as Leishman-Donovan bodies (L.-D. bodies) after the names of the discoverers, and they are classified under the genus Leishmania. The diseases caused by them are known as Leishmaniasis, kala-azar being called visceral leishmaniasis, and oriental sore and espundium mucocutaneous leishmaniasis.

There are four species belonging to the genus Leishmania:—

(*i*) Leishmania donovani, Ross, 1903 (Syn. L. infantum), parasite of—(*a*) Indian kala-azar and,
  (*b*) Infantile kala-azar of the Mediterranean littoral.

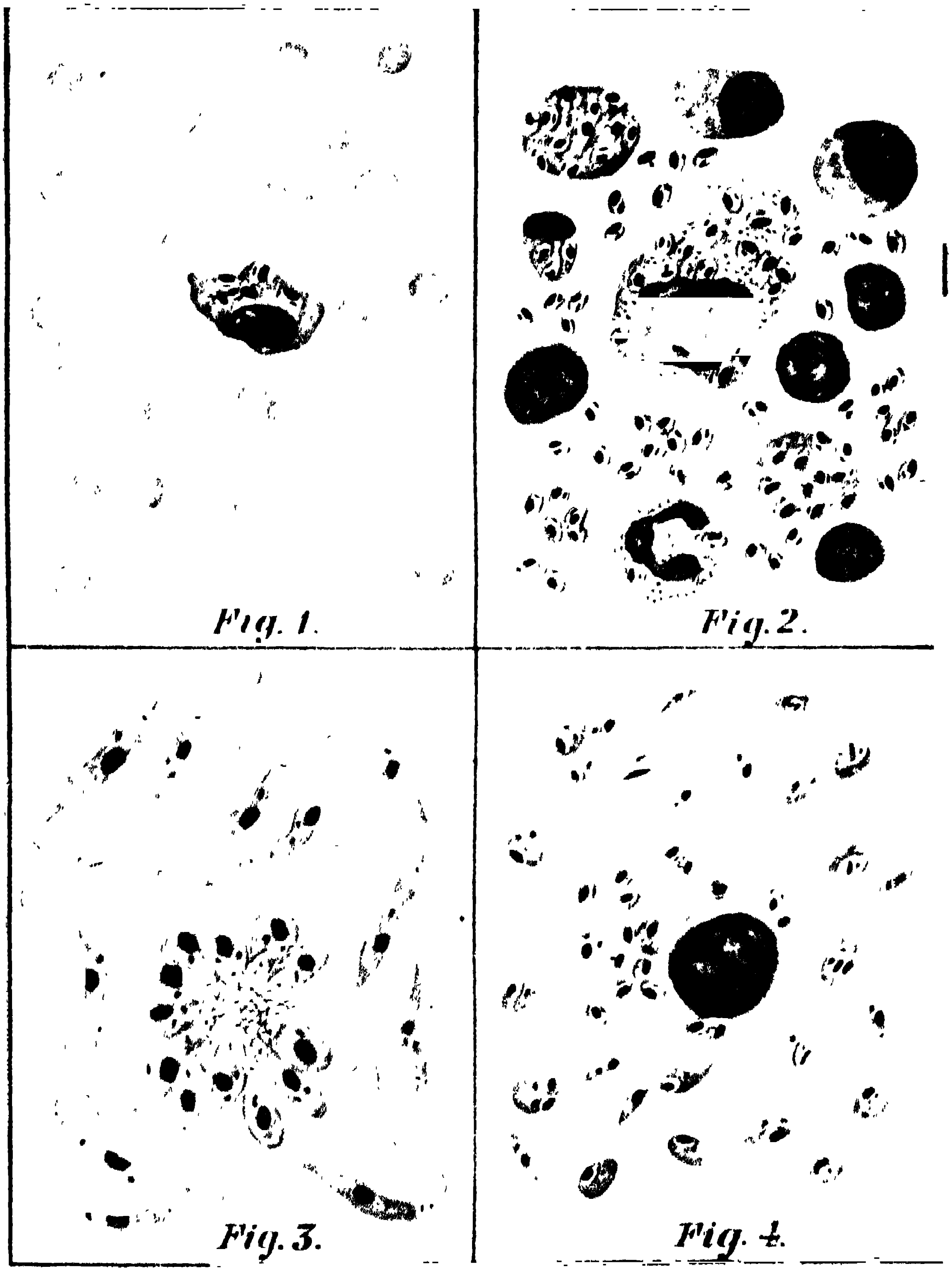
(*ii*) Leishmania tropica, Wright, 1903, parasite of oriental sore.

(*iii*) L. braziliensis, Vianna, 1911, the parasite of espundium affecting the mucous membrane of mouth and naso-pharynx. May also affect skin.

(*iv*) Parasite of canine leishmaniasis.

Of these, the first three are of human importance. Formerly, it was supposed that the infantile kala-azar of the Mediterranean littoral

Plate No. III



Leishman-Donovan bodies

Fig 1 From peripheral blood

Fig 2—From spleen puncture

Fig 3- From culture

Fig 4 From Oriental Sore

Face Page 200

was caused by a parasite of separate species—L. infantum. This was supposed from the observation that Indian kala-azar was an affection of adults only, and that children were rarely infected, whereas just the opposite condition was present in the Mediterranean littoral. Further, the parasite of infantile kala-azar can infect the dog, whereas that of Indian kala-azar cannot.

Recently, it has been observed, that kala-azar in India is not rare in children, and dogs can be infected with material from Indian kala-azar. Further, the parasites causing the adult and infantile kala-azar are practically identical, both morphologically and culturally. Also, from the clinical features of the cases, it is evident that infantile kala-azar can no longer be maintained as a distinct form: L.

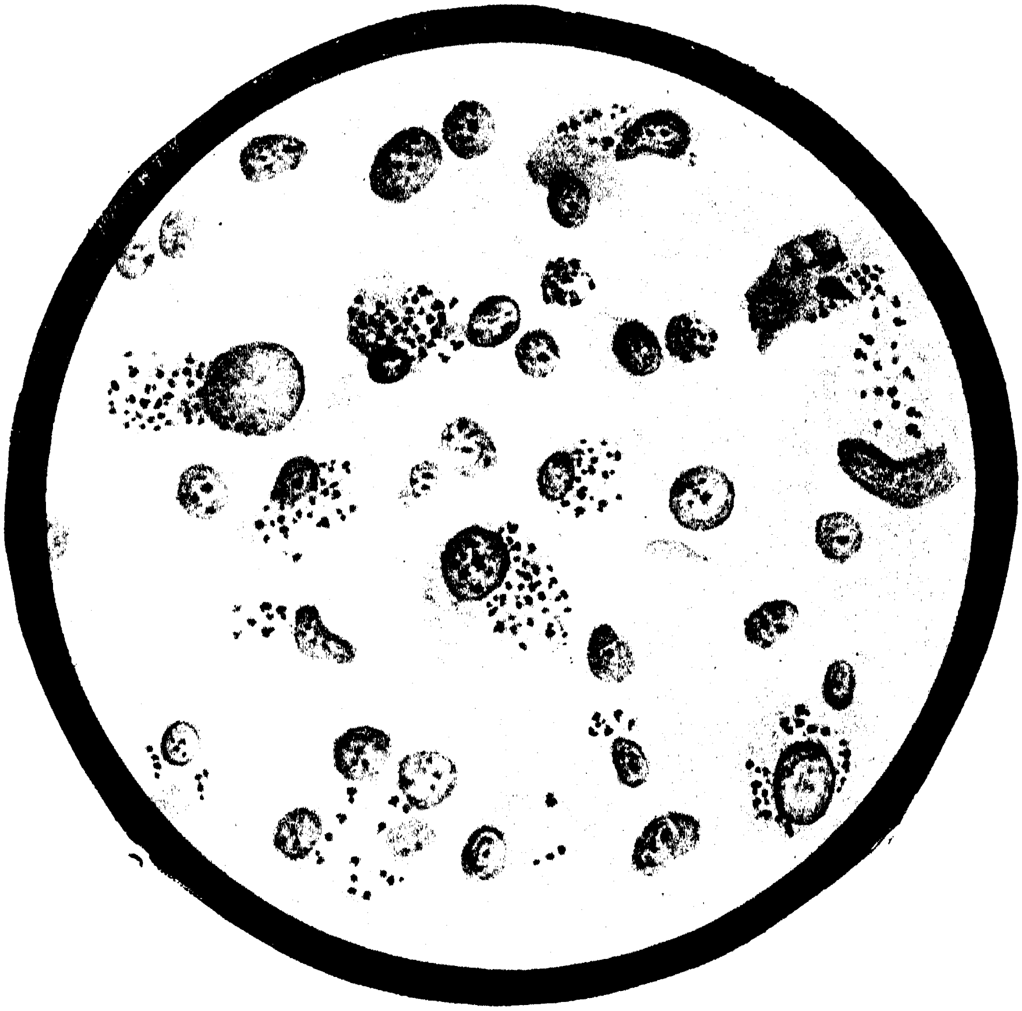


Fig. 67. L.-D. bodies in section of spleen.
(From a specimen of Dr. Monindra Nath Dey.)

donovani and L. infantum are to be considered as belonging to the same species. The same thing holds good for the Sudan species of kala-azar parasite of Archibald.

L. donovani is present in the cytoplasm of the endothelial cells of the spleen, liver, bone marrow, kidneys, supra-renals, testes, and other organs. They are found in the peripheral blood and inside the leucocytes or large mononuclears only in small number.

Morphology.—L.D. bodies are minute oval or torpedo-form parasites measuring 2-4 μ in length and 2 μ in breadth. It consists of a definite cell wall with a cytoplasm staining pale blue with Leishman's stain. There are two chromatin masses: one large, the *trophonucleus,* which is round and placed near the margin, the other, *rhizoplast,* which is smaller, more or less rod-shaped, stains deep-red with Leishman's stain, and placed at a right angle or tangentially to the macronucleus. The rhizoplast, strictly speaking, represents two constituents—the parabasal body and the rudimentary root of the future flagellum. Anterior to the rhizoplast, there is a small vacuole which takes eosinophile staining with Leishman stain, and is hence called "eosinophile vacuole." In a smear from spleen, we find large numbers of parasites either inside the cells or free. The parasite is always intracellular. The free parasites in the smear are due to mechanical rupture of the parasite-laden endothelial cells during preparation of the smear. That they are intracellular, can be well demonstrated in section of the spleen, appropriately made and stained.

So long as they are present in the tissue, there are no flagella; but flagella appear when they are grown on artificial culture medium.

The morphological character of the L.-D. bodies in culture resembles a herpetomonas, the flagella and the parabasal body being on the same end, *viz.*, the anterior end. During movement, the flagellum proceeds in front, drawing the parasite along with it, like the horse dragging the cart. There is no undulating membrane.

In culture, they often collect together with all their anterior ends in the centre, the whole collection being called agglomeration. There may be secondary groups of agglomerated organisms round the primary ones.

Multiplication.—Takes place in the endothelial cells by binary fission, until the cells become packed up with the parasites, and rupture takes place. The parasites, thus liberated, will enter fresh endothelial cells, and go on multiplying. The flagellate form divides also by binary longitudinal fission.

Cultivation.—The culture media used for the cultivation of L. donovani are the following:—

(1) Rogers' acidified citrate solution.--Citrated salt solution, slightly acidified with citric acid.

(2) Nicolle-Novy-MacNeal medium, commonly known as the N.N.N. medium.

Take of agar 20 grm., sodi. chloride 9 grm., water 1 litre, sterilize in an autoclave and distribute into test-tubes, each containing about 1 c.c. of the medium. Sterilize each and put in water bath at 60°C, add to each twice its volume of rabbit's blood obtained from heart puncture, then allow to solidify and keep them in cold incubator (22°C) so as to get the maximum amount of water of condensation, which is essential for the growth. Reject the tubes which show presence of contamination.

(3) Row's hæmoglobinised saline medium.

Take 10 c.c. blood from rabbit's heart, or arm vein of man, defibrinate it, and add 10 volumes of sterile distilled water to lake the cells. One volume of this laked blood is added to two volumes of sterile 1.2 per cent. saline.

There is a great deal of controversy about the various ingredients of the media necessary for the adequate development of L.-D. bodies. Some maintain that intact red blood cell is essential for the growth, whereas others find hæmoglobin as essential, while others again observed Leishmania to grow in media containing no hæmoglobin at all.

About the serum, it is generally believed that human serum is detrimental to the growth, and hence, rabbit's blood is preferred. Various workers used the blood of different animals with varying results.

Opinion also differs about the percentage of sodium chloride to be used in the media; and it is believed that the hydrogen-ion-concentration of the medium is also a great factor for the successful growth of the organism.

Contamination of the culture tubes invariably leads to destruction of the flagellates. With a view to finding out which factor of the bacterial contamination leads to the destruction of the flagellates, Cornwall and Menon performed some experiments, and found that, when soluble products of bacterial metabolism were added to the culture tube, the flagellates continued to multiply. No inhibition of growth was observed when autolysed bodies of dead bacteria were added to the N.N.N. culture tubes. Hence, it is concluded that the destruction of the flagellates, due to contamination of bacteria in the culture tube, is primarily due to exhaustion of the available nutriment.

Laveran found cultures of L. donovani contaminated by a fungus which grows luxuriantly in the tubes. The Leishmania were not destroyed and continued in good culture. In order to get rid of the fungus, into each tube were introduced 5 drops of a 1% caustic soda solution. Four days later, the tufts of fungus disappeared and subculture yielded pure cultures of L. donovani.

Spagnolio found that M melitensis did not kill the L.-D. bodies in cases of contamination of the culture tubes.

Temperature and Growth of L. donovani.—Blood heat, which is the optimum temperature for most organisms, is not suitable for the Leishmania flagellates. Rogers, in his attempts to cultivate them at blood heat, found that they die out in a day or two. On reducing the temperature to about 22°C, he found that the organisms multiply rapidly, increase in size, and develop into the actively motile flagellate form. All the other workers agree that 22°C is the optimum temperature for the growth of L.-D. bodies. Attempts have been made to cultivate them anærobically, but, with negative results.

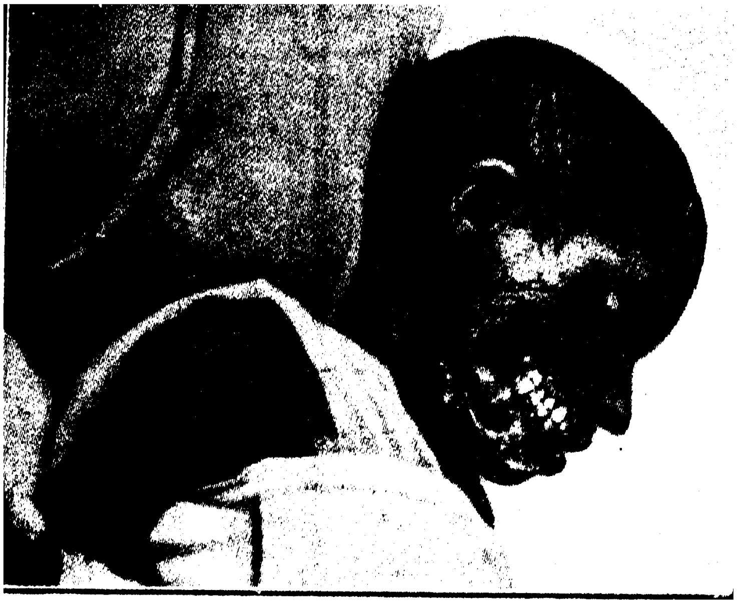


Fig. 68. Cancrum oris.
(From "A Treatise on Kala-azar"—Brahmachari).

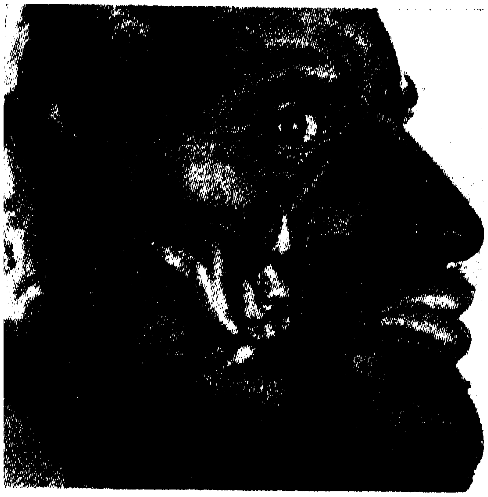
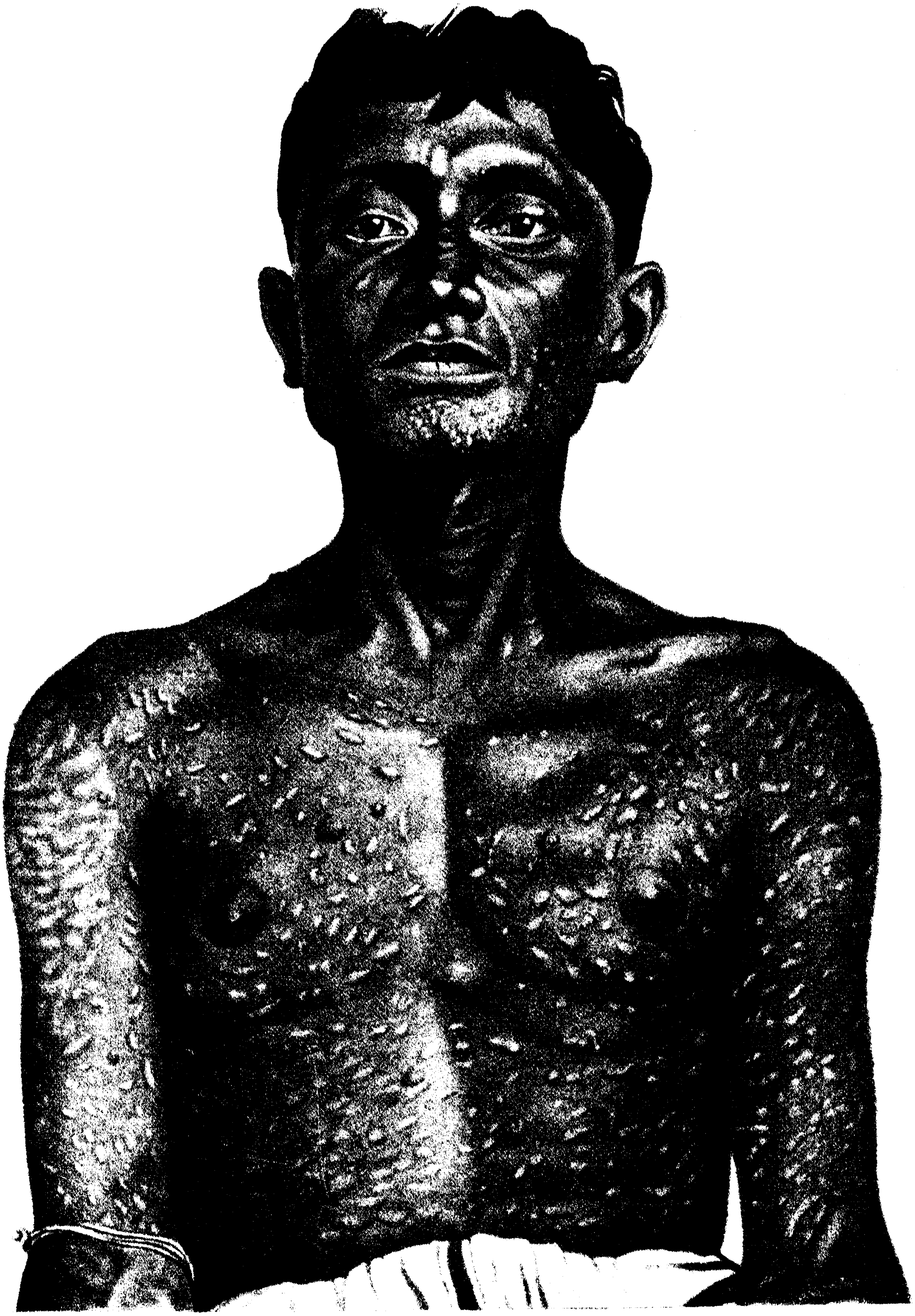


Fig. 69. Cancrum oris following Kala-azar.

Subculture—Nicolle obtained 100th and 56th subcultures of L.-D. bodies from human cases, 61st and 58th passages of canine kala azar, and 100th subculture from oriental sore which had been kept from Nov. 1909 to June 1914. All these forms grew readily as little transparent colonies on the surface of agar, the flagellates having undergone no change in morphology during this time. He also isolated 3 strains of Leishmania from cases of kala-azar and maintained for 291, 241, and 59 subcultures.

The method which has been most successful in our laboratory is that of Row. Take about ½ to 1 c.c. of blood from the vein and put in 20 c.c. citrated saline and mix thoroughly. Put this in incubator (22°C) for 24 hours, after which take the sediment with sterile pipette and inoculate N.N.N. media. Growth is obtained on the 4th or 5th day. In N.N.N. medium, one finds, according to



Dermal Leishamniasis

(From Treatise on Kala azar----Dr. Brahmachari.)

the stage of development, various forms of the parasite—unaltered, pre-flagellate form, globular or elongated flagellated form, post-flagellate form, "O" or "torpedo," aflagellated form, and other various degenerated forms.

Difference between L. donovani and L. tropica

	L. donovani.	L. tropica.
1.	Optimum growth at 22°C. killed at blood heat	Can grow at blood heat .
2.	Contamination of culture rapidly kills the organism.	Can grow in presence of contamination.
3.	Very few susceptible animals for inoculation.	Animals infected readily, especially dogs and monkeys.
4.	Transmission unknown (sandfly ?).	Transmitted by sandfly—Gecko acting as reservoir.

Animal Inoculation

Laboratory animals, *e g*, monkey, dog, rat, mouse, etc., are infected with great difficulty, and that only after injection of a fairly large dose of the organism from fresh spleen juice. Injection of the flagellate from culture gives still more unsatisfactory results. The only susceptible animal is the Chinese hamster, which again has shown less susceptibility when brought down to Calcutta.

Transmission Problem of Kala-azar

Since the discovery of the Leishman-Donovan bodies, the fact that Kala-azar is a distinct infectious disease was settled. Again, on account of its similarity with malaria, more specially both of them being caused by systemic infection by the respective parasites, the former being transmitted by biting insect mosquito, it was assumed naturally that kala-azar was also transmitted by a biting insect, and hence the common blood-sucking insects (mosquitoes, bugs, fleas, sandflies, ticks and some flies) were looked upon with suspicion.

It has long been observed that kala-azar is a house and family disease Further, Rogers subsequently observed that, in the process of segregation in Assam, a new cooly line separated from the old infected one by only a distance of 300 yards, was sufficient to escape infection for several years. From this, it was supposed that an insect which can migrate easily a distance of over 300 yards is probably not the transmitting agent.

These facts, and that the *bed-bug* can be very easily transported from one place to another, that it can resist prolonged fasting, and that it ingests a fair quantity of blood with each feed, naturally accuse this insect—the bed bug—as the transmitting agent.

Numerous enthusiastic workers—notably Patton, Mrs. Adie, Cornwall, and others—worked to bring out the exact part the bed-bug was playing in kala-azar. Patton, working in Madras, had greater opportunity to observe feeding experiments with blood of patients, as the Madras cases peculiarly show large number of parasites in the peripheral blood. He observed that the bug sucks up the blood with the leucocytes containing L.-D. bodies which developed into flagellates. It is not uncommon to find the gut of the bug literally packed up with the flagellates after the feed.

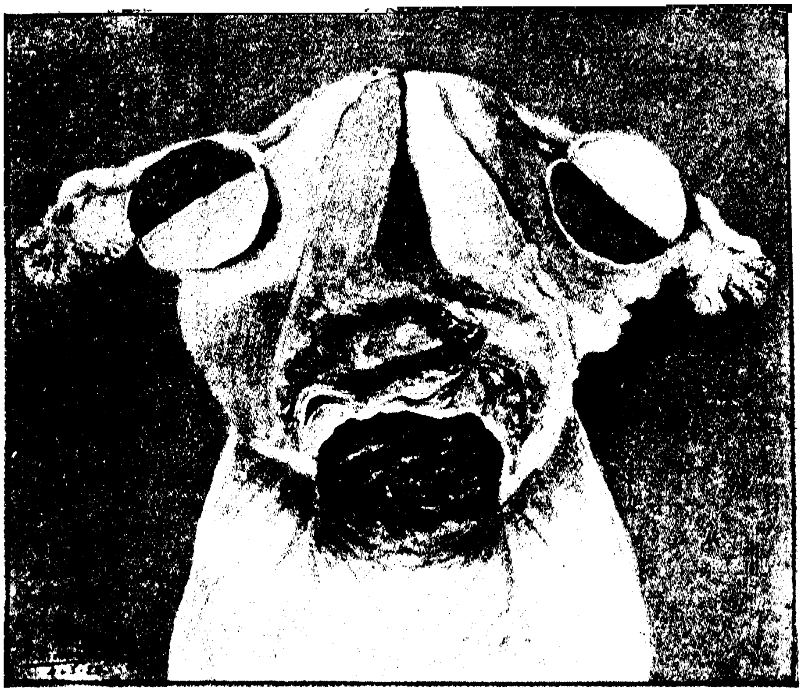


Fig. 70. Sloughing of Cervix uteri in a case of latent Kala-azar. (From a specimen of Dr. Manindra Nath Dey.)

Mrs. Adie went further and observed the L.-D. bodies actually entering the cells of stomach of the bug with subsequent development and multiplication. She also found the parasites in the salivary glands of the bug. Thus, from the observation of the two workers, it is found that the L.-D bodies enter the stomach of the bug, multiply in the lumen, then enter the cells of the stomach, develop further and, to complete the cycle, find their way into the salivary glands, forcing to the conclusion that "this is the final proof that bug is the carrier of Leishmania donovani." From the perusal of the findings of other workers, we observe that other flagellates, *e.g.*, rat typanosome, L. tropica, Leptomonas pulicis, Crithidia ctenocephali, Herpetomonas muscæ domesticus, and numerous other flagellates, which are certainly not transmitted by the bug, also develop in the bug as typical flagellates, and survive longer than the Leishmania donovani.

Thus, it may be assumed that the development of Leishmania donovani in the stomach of the bug is of a similar nature as those of the flagellates of the dog flea and house fly just mentioned. Further, the presence of a large quantity of slowly digesting blood in the stomach of the bug acts as a culture tube, and does not mean any stage of the life cycle.

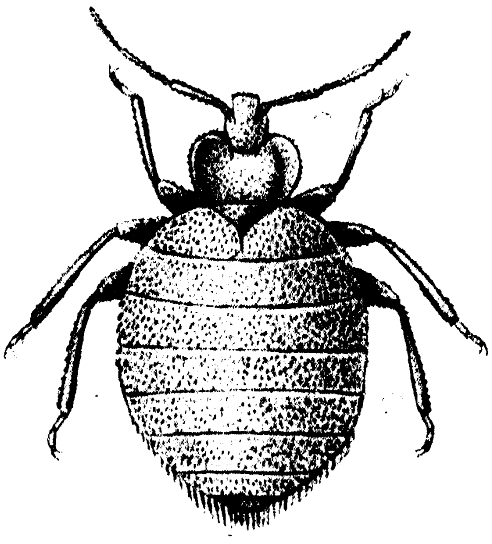


Fig. 71. Bed bug

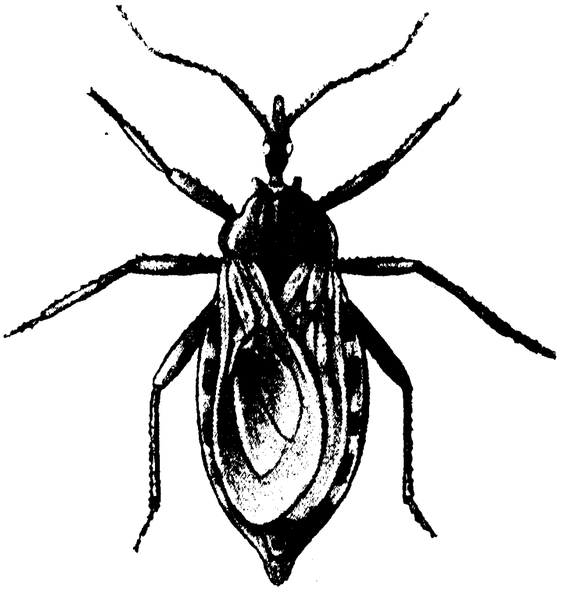


Fig. 72. Conorrhinus.

As regards the observations of Mrs. Adie, none has been able to confirm them up till now Moreover, her observations being made on dead bugs kept in saline for a few days, in the opinion of the authorities, the accuracy of the observation was questionable: probably they were not L. donovani at all.

Again, from Patton's observations, we find that if the bug is fed upon human blood for the second time, the flagellates developed after the first feed are quickly destroyed This seems to go against the bug theory.

Bed-bug is practically cosmopolitan, but kala-azar has got a limited distribution Bombay can be mentioned as an instance where kala-azar is scarcely found, but bugs abound, and Bombay is exposed to attack from bugs transported from different parts of India through bed or furniture.

The results of the workers can be summarised, as concluded by Wenyon, "that the bed-bug, though a suspected agent, still awaits the final verdict, and that, unless more tangible and convincing evidence is forthcoming, the jury will certainly disagree. Every credit is due to the prosecution for their most careful and exhaustive inquiries which have extended over many years and which have been stimulated by a desire to rid the world of a harmful parasite; but the elusive bug, which has been acquitted of so many charges, has so far escaped sentence in the case of kala-azar also."

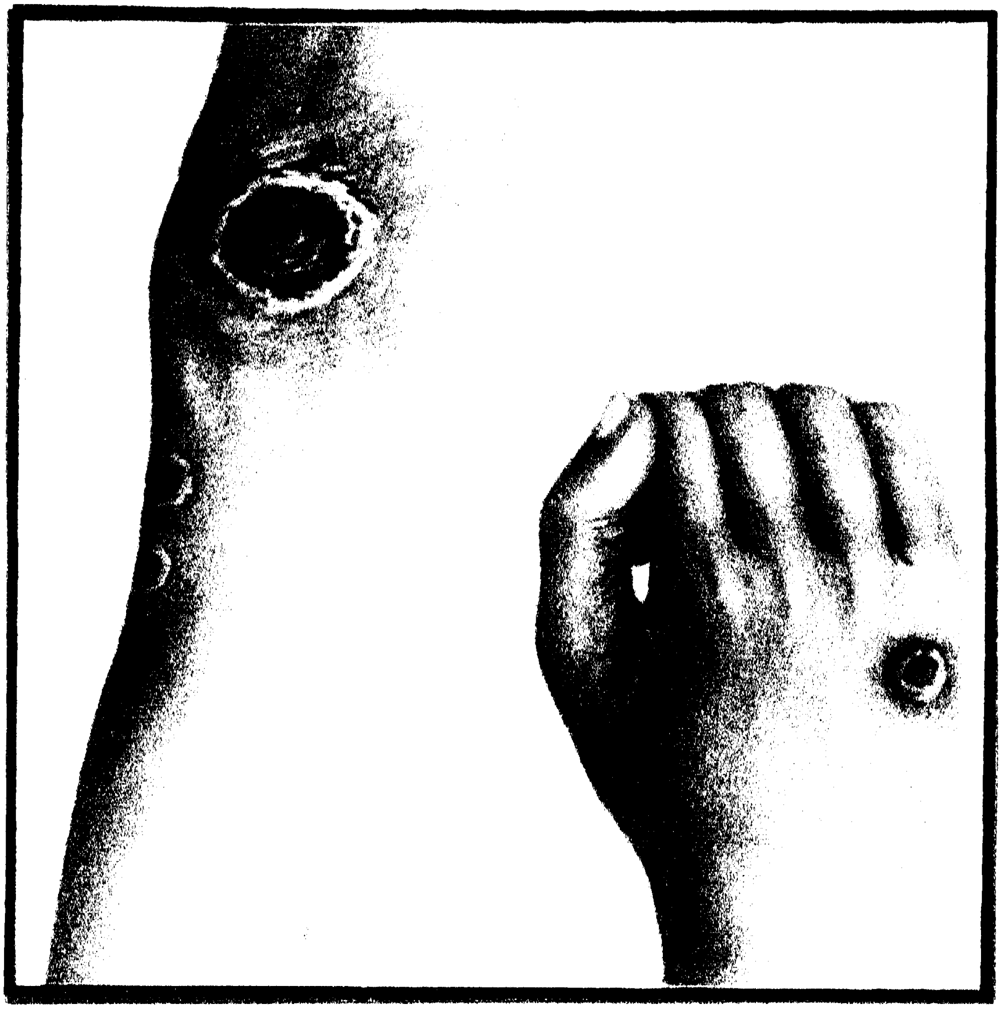
The *riduvid bug conorrhinus* is regarded to be the transmitting agent of some form of trypanosomiasis, and hence it is supposed also to transmit Indian visceral leishmaniasis. But Patton finds that it does not ordinarily feed on man, and the organism in this bug rapidly degenerates and never flagellates.

Next comes the *flea*. Numerous workers are working with the dog flea—Ctenocephalus canis—but no substantiating evidence has yet been found. Basile, working in the Mediterranean area, claims to have observed the development of Leishmania donovani in the flea, and that the infected fleas are able to transmit the disease to healthy dogs. Unfortunately, corroboration is lacking. Further, as the dog flea often suffers from natural infection with flagellates, it is difficult to differentiate L. donovani from the natural herpetomonas. Moreover, dogs in the Mediterranean area are liable to have a latent infection of Leishmania.

Mosquito.—Franchini observed various forms of L.-D, bodies in the gut of anopheles after feeding them upon artificial culture of organisms. Wenyon also observed L. tropica developing into flagellates in the gut of stegomyia fasciata after feeding upon the non-ulcerating form of oriental sore. In view of these observations, as well as the development of L. tropica in bed-bug, Wenyon, Mesnil and others think that "the parasite of the group Leishmania will develop, to a certain extent, in the intestines of many insects, provided that they find there a suitable pabulum rich in blood, and does not necessarily mean that the insect in question is the natural host of the parasite."

Sandfly (Phlebotomus argentipes):—Naturally, along with the other insect "suspects," sandfly was also called upon to undergo the vigorous laboratory trials, but on account of very poor evidence, it was acquitted, until Knowles and others appealed for retrial. These workers found that Calcutta can be divided arbitrarily into portions, where kala-azar is endemic, and other places, *e.g.*, Northern Calcutta, which, in spite of large number of kala-azar patients coming constantly from the muffusil for treatment, still remain non-endemic. The comparative survey of these endemic and non-endemic areas suggested to them that the sandfly might be the responsible transmitting agent of this disease. It was then found that the geographical distribution of sandfly in India is identical with that of kala-azar. As good luck would have it, the experimental feed on kala-azar patients showed very heavy infection with herpetomonad form in the fore-gut and mid-gut of the insects at the third to fifth day, whereas a very large number of control sandfly, either wild or bred in the laboratory, with or without blood-feed on patients suffering from diseases other than kala-azar, showed invariably a negative finding, proving that no natural herpetomonad infection is present in the sandfly, and that the herpetomonad forms, found after the feed on kala-azar patients, were nothing but L. donovani.

Plate No IV.



Dermal Leishmaniasis

From a Panjabi Musalman who noticed a red papule at the root of his right little finger about 10 or 12 days from the date he tried the strength of his wrist with one of his class mates who had some ulcers on his right hand. The other ulcers come up one by one from auto-infection

(*From Calcutta Medical Journal.*)

After very elaborate research on sandfly the Kala-azar Commission (Christophers, Shortt, Barraud and Craighead) and the Calcutta workers (Knowles, Napier, Smith and others) came to the conclusion that the sandfly—Phlebotomus argentipes—is the true vector of kala-azar.

Considering the fact that Knowles rightly puts—" It is a history of almost of 20 years of wasted efforts of individual workers starting off with the highest hopes and ending in despair; of false starts and erroneous conclusions; of acute controversies and the flow of much ink; of wasted individual effort and the absence of co-ordinated enquiry; finally, however, of really concerted effort with successful results,"—we may hope, along with Knowles, "as one writes even now the end is not yet, but it is not unreasonable to hope that it is not very far off." Only the further will answer that!

Patton and Mackie, after various cultures and animal experiments, exclude other blood-sucking insects, *e.g.*, mosquito, flea, lice, tic, sandfly, etc. Inspite of all these workers, science is still very uncertain in the finality. The main reason is that the crucial test lies on two points. successful inoculation (1) to a laboratory animal, and (2) to man

As regards the former, there is still a great want for a successful laboratory animal: monkeys, flying-foxes, hamsters and white mice are found to be susceptible. About successful inoculation, again, the difficulty is greater. Inoculation with flagellates from culture is mostly unsuccessful. If one wants to inoculate successfully, he must choose the material from a patient suffering from the disease. Hence, it is most likely that the flagellate stage is not the infective stage. The infective stage is the leishmania form as found in the tissues of man.

Further, subcutaneous inoculation with the leishmania from spleen puncture material to a susceptible animal, *e.g.*, monkey only leads to a localised nodule, and rarely, a systemic infection. They can be infected intraperitoneally. Hence, it is natural to doubt whether the subcutaneous inoculation is the rule in nature to cause infection

Thus, workers are absolutely handicapped about the successful inoculation to an animal causing visceral leismaniasis.

To sum up the transmission question, we find the two possibilities—(1) *via* skin, through biting insect, and (2) *via* alimentary tract, through feeding.

(1) As regards the former, we often observe flagellates develop in the gut of the insect after an experimental feed. Those insects may have natural infection with flagellates indistinguishable from L. donovani, or the gut acts as a culture tube. To consider the other end of the chain: Inoculation experiments with flagellates from cultures show that probably the flagellate stage is not the infected one. No definite experimental infection of laboratory animals has been demonstrated by biting with infected insect. Again,

inoculation of the fresh spleen puncture material subcutaneously to a laboratory animal leads only to a localised nodule, and not to a general infection; so, it is rather improbable to lead to visceral lesion by the biting of an insect.

(2) To consider the other possibility, *i.e.*, transmission through the alimentary tract, we find that there is a close relation between kala-azar and bad conservancy.

To compare Calcutta with its suburbs, although Calcutta is constantly being exposed to infection from imported cases from moffusil, still it is very rare to find a case of kala-azar occurring in Calcutta itself (Northern Calcutta), whereas the suburbs are intensely infected, and the only difference between these two places is probably the improved drainage and water supply Is it then the fact that improved conservancy prevents the occurrence of kala-azar and *vice versa?*

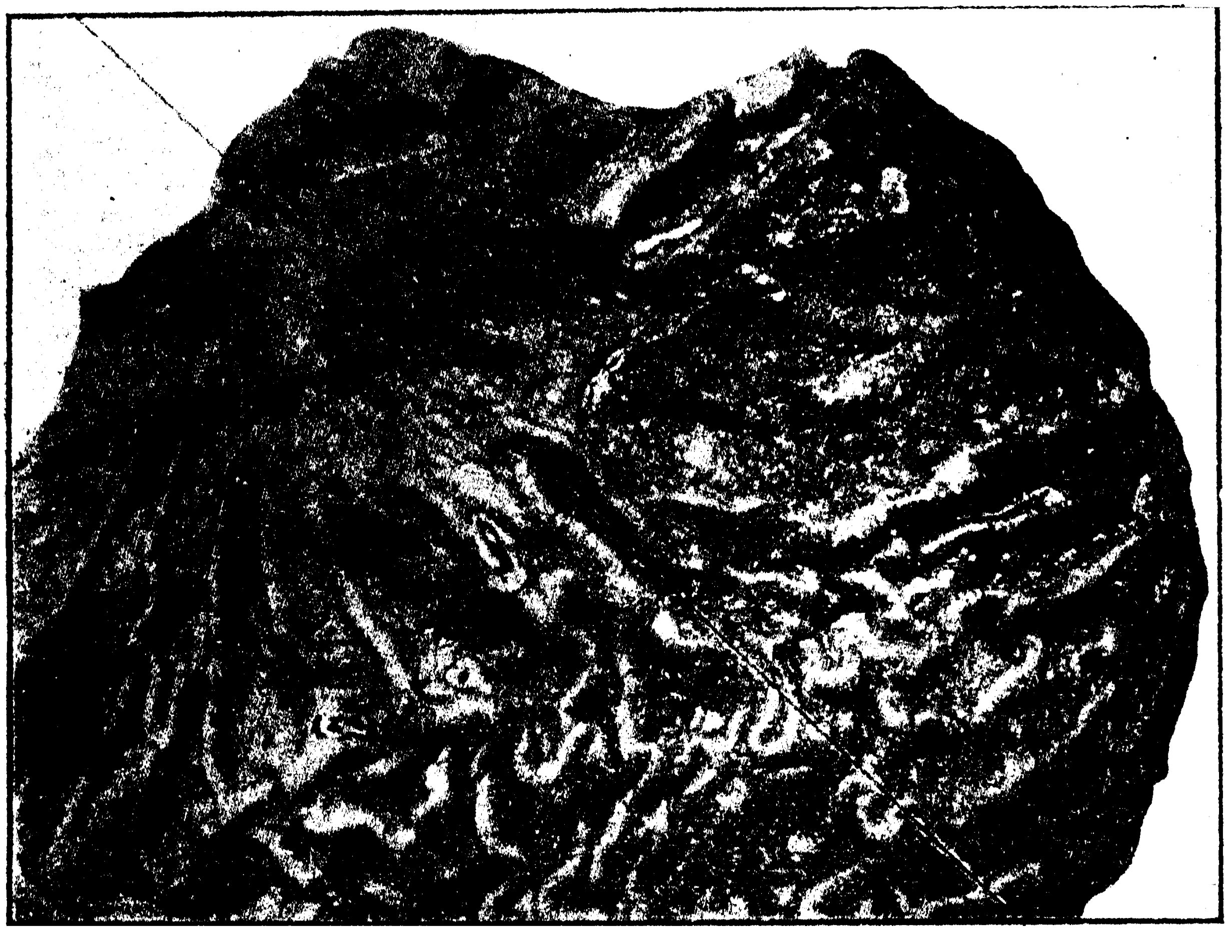


Fig. 73. Leishmania ulcers of the stomach in author's case of latent Kala-azar.

Knowles records a case where a Municipal sweeper in Nowgong contracted kala-azar, and apparently his only contact with the disease was through the medium of conservancy work. He further records from McCoombie's observations, that, of the staff of the special kala-azar hospitals in Assam, the only members who contracted the disease, were three sweepers.

He examined washed mucus from dysenteric stools of **kala-azar** cases and found bodies similar to L.-D. bodies, but they were most atypical, and suggests that "the possibility of direct fæcal transmission from person to person by contamination of hands, clothings, etc., of kala-azar cases suffering from dysentery should not be lost sight of."

Marian-Perry has observed Leishmania in the mucous membrane of jejunum, which has been confirmed by Mackie.

The present writer has observed the occurrence of Leishmania in ulcers of stomach in a case of Kala-azar (*Cal. Med. Jl.*, Sept. 1923). (*See Fig.* 73).

Kala-azar cases are very liable to dysenteric complications, and hence it might be supposed that the infective organisms leave the body with the fæces.

To consider the other end of the chain—namely, how it enters the body —it has been experimentally observed by Cornwall, Archibald and Knowles that feeding monkeys and other animals with emulsion of spleen from post-mortem of a recently-dead kala-azar case, leads to general Leishmaniasis and subsequent death.

Thus, we find that, after about 20 years' laborious researches by eminent workers, science is still not in a position to say definitely whether the insect is, or is not, the vector. We are at present absolutely in the dark about the transmission problem of the disease.

Further, experimental inoculation of the susceptible animal by biting of these infected insects always leads to unsuccessful results, the probable reason being that the flagellate stage may not be the infective stage.

(*b*) Fantham and Porter's suggestion that the natural flagellates of insects are the "leishmania in the making" is still unsettled.

Pathology of Kala-azar

Spleen.—enormously enlarged, with thickened capsule. Pulp is increased and congested. Histologically, great proliferation of the clasmatocytes loaded with parasites takes place. Reticulo-endothelial system shows proliferative changes. *Liver and Bone Marrow.*—Show similar changes. *Blood.*—Anæmia, leucopenia (less than 2000 leucocytes per cmm.); the relation of R. B. C. to W. B. C. is 1000: 1 or even 4000: 1; great reduction of polymorphs but relative increase of small and large mononuclears. There is progressive emaciation.

Dermal Leishmanoid.—L.-D. bodies found in the smear from nodules.

Diagnosis.—*See* laboratory methods.

TRYPANOSOMES

This organism is widely distributed amongst the vertebrates and the invertebrates, as well as in the plants. In Calcutta, it is very common in rats, almost every rat harbouring trypanosomes in the blood.

There are three species which are pathogenic to man. They are:—

(1) T. gambiense—causes *sleeping sickness* (Africa).
(2) T. rhodesiense—causes *sleeping sickness* (South Africa).
(3) T. cruzi—causes *Chagas disease* (South American form).

Trypanosomes infecting animals are:—

(4) T. lewisi of rat. (5) T. evansi of horse, causing *surra.* (6) T. brucei of oxen, causing *nagana.* (7) T.equiperdum of horse, causing *dourine.* (8) T. equinum of horse, causing *Mal de caderas.* (9) T. theileri of cattle.

There are hundreds of other trypanosomes infecting animals.

T. GAMBIENSE

(Dutton 1902)

The distribution is wide-spread in Africa. They occur in the blood of human beings but are more readily found in the C. S. Fluid or on puncture of an enlarged gland.

Morphology.—Size varying from 15 μ to 30 μ in length, and 1·5 to 2·5 μ in breadth. It is spindle shaped and flat, one end is blunt, and the other, the anterior end, is pointed and provided with a flagellum. There are two nuclei, one macronucleus, also called trophonucleus (because it regulates nutrition), which is round, large, and placed centrally; the other the parabasal body (micronucleus), which is a small dot or rod and stains deeply with Leishman stain, is placed at the blunt posterior end opposite to the flagellum (*cf.* herpetomonas). Posterior to the parabasal body lies a vacuole, called the eosinophile vacuole, and behind the vacuole there is a small basal granule, from which the flagellum originates and passes along the body of the parasite, at the same time raising the cell wall to form the undulating membrane, and terminates as a free flagellum

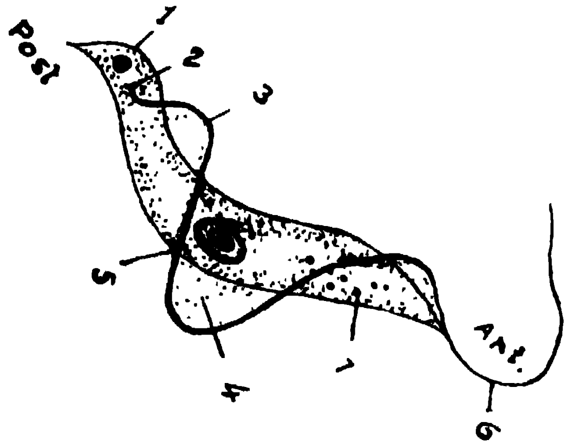


Fig. 74. Schema of Trypanosoma
1. Basal granule. 2. Parabasal body. 3. Marginal flegellum. 4. Undulating membrane. 5. Nucleus. 6. Free flagellum. 7. Chromatoid granules.
Ant—Anterior extremity
Post—Posterior extremity

at the pointed end. The parabasal body and the basal granule (blepharoplast) together are called the kinetoplast. There is much difference of opinion as regards the polarity of the parasite. The trypanosome moves forward with the free flagellum in front, so that the flagellar end is the anterior end. In addition to these structures, numerous granules appear throughout the parasite. They are present in the blood of man. Unlike the parasites of malaria and kala-azar, which are found in the blood corpuscles (red and white, respectively), the trypanosomes are found in the plasma. They are also found in the cerebro-spinal fluid, and it is peculiar to note that, in the early stages, they are more numerous in the blood than in the cerebro-spinal fluid, whereas, in the late stages (sleeping sickness stage), they are found to be more numerous in the cerebro-spinal fluid than in the blood. It has been found recently that the path of infection in trypanosomiasis is through the lymphatics rather than through the blood circulation.

Multiplication.—By binary fission. The kinetoplast divides first.

Cultivation.—Like Leishmania they grow in the *water of condensation of blood agar* (Nicolle-Novy-Neal medium—briefly N. N. N. medium). In culture, they are often seen aggregated together as clumps, the process being known as agglomeration. Occasionally clusters of secondary agglomeration are observed round the primary one.

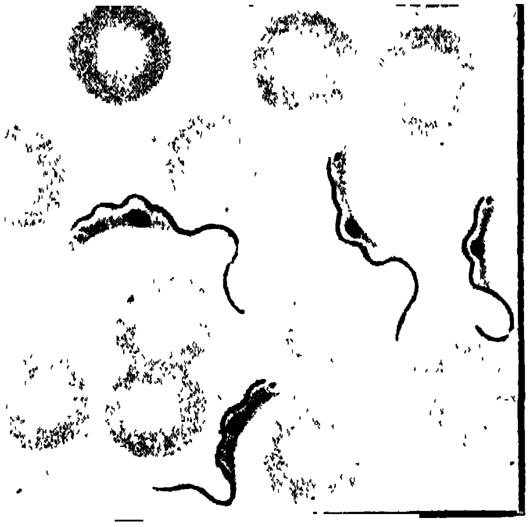


Fig. 75. Trypanosoma gambiense.

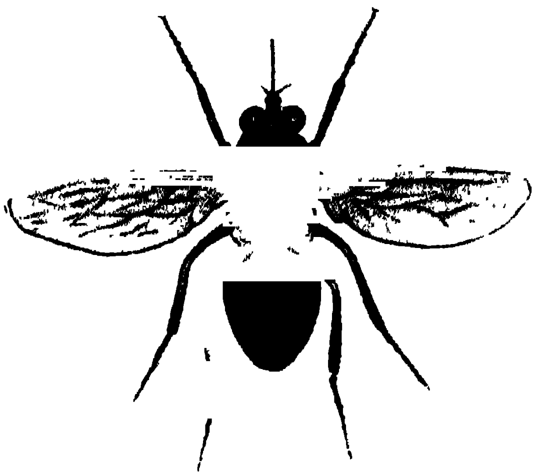


Fig. 76. Tse-tse fly.

Biology.—The serum of an animal which has recovered from an infection of trypanosomiasis shows the presence of immune bodies and often gives complement fixation tests. Laboratory and domestic animals are susceptible.

Transmission takes place through the blood-sucking fly, Glossina palpalis, commonly known as *tse-tse fly*. These flies live near the edge of water, explaining thus the distribution of the disease along banks of rivers and edges of lakes, and the liability of fishermen to the disease. They multiply in the stomach of the fly and migrate to the proventiculus on the 10th-12th day and then to the salivary glands. Here the cycle is completed in a further period of 2-5 days when the infective forms for man are produced.

Pathology.—The lymphatic glands and the nervous system are the chief seats of the lesion. The lymphatic glands, specially of the cervical and submaxillary regions, are enlarged, congested and often show hæmorrhages. The central nervous system shows, histologically, inflammatory changes of the brain and hæmorrhages with extensive perivascular round-celled infiltration, exudation of lymph and increase of cerebrospinal fluid.

T. RHODESIENSE

This is the parasite of sleeping sickness of South Africa. It resembles T. gambiense. It has no special relation with water edges. Unlike T. gambiense, T. rhodesiense is readilv transmissible to laboratory animals also, while to rats it is virulent from the very beginning, and in this host a change in the position of the nucleus takes place.

T. CRUZI (SCHIZOTRYPANUM CRUZI)

This is the causative organism of South American form of trypanosomiasis, called Chagas disease. The parasite is intermediate between trypanosoma and leishmania. It is transmitted by the reduvid bug, Triatoma of Conorrhinus megistus, and not by a tse-tse fly. In man multiplication occurs in the striated muscle fibres and neuroglia cells of the brain, and that in the Leishmania form.

GENUS TRYPANOPLASMA

This resembles trypanosoma, except in that it has two flagella, one at each end.

CHAPTER XVI

SPOROZOA

This includes all organisms which multiply by means of formation of spores. They are all parasitic and get ample nutrient elements from the tissues of the host. By adaptation they have been deprived of all forms of independent power to move from place to place or to capture food.

Classification :—

A. Class Hæmosporidia.—This is divided into the following genera :—

(*i*) Plasmodium—3 species, *viz.*, P. vivax, P. malariæ, P. falciparum.

(*ii*) Hæmoproteus (Halteridium)—affects red blood cells of birds, and especially the pigeons.

(*iii*) Piroplasma—affects man and lower animals. Causes human Piroplasmosis—Oroya fever, Rocky Mountain fever, which is transmitted by tick.

(*iv*) Hæmogregarine affects animals.

(*v*) Leucocytozoon.

B. Coccidia.

C. Rhinosporidia.

D. Sarcosporidia.

Plasmodia (The Malarial Parasites)

They always exist as parasites, and, as they belong to the group hæmosporidia, they are parasites of the blood. In the blood they live inside the red corpuscles, differentiated from the hæmoflagellate leishmania in leucocytes and trypanosoma in plasma.

History of Discovery.—The parasites were first discovered by Laveran in 1880. The 'flagellation' of the crescents were first observed by Laveran and then by other Italian workers—Marchiafava, Celli, Golgi, Bignami and Bastianelli. In 1894, Manson suggested that it is transmitted by the mosquito and that the cycle of development takes place in the mosquito. This was discovered by Ross in 1895, with a bird parasite—Plasmodium precox. Grassi observed the same with human malaria and demonstrated the transmission by anopheles. MacCallum discovered the process of sexual fertilization of the parasite of bird malaria, the Hæmoproteus columbæ, in 1897. Grassi, Bignami and Bastianelli discovered the complete development cycle of P. falciparum, P. vivax and then of P. malariæ in anopheles maculopennis in the year 1899 and Grassi described in full detail the whole of the cycle of sporogony of all the malaria parasites of man in 1900.

There are three species which are distinct, but more or less alike in morphology and life history. They are—

(*i*) P. vivax—parasite of benign tertian form.
(*ii*) P. malariæ—parasite of quartan form.
(*iii*) P. falciparum or Laverania malariæ—parasite of malignant tertian form

PLASMODIUM VIVAX

(Grassi & Filetti 1890)

(*Parasite of Benign Tertian Malaria*)

Morphology and Life History.—Asexual cycle. It starts its life as a tiny ring consisting of a cell wall enclosing the cytoplasm (stained blue with Leishman's stain) and a chromatin dot (stained deep red with Leishman). This ring-shaped parasite first of all remains attached to a red blood corpuscle, and subsequently enters into it. The ring grows bigger with the formation of a vacuole inside it, which distends the parasite, which in its turn grows thicker. The ring measures about 3 μ ,*i.e.*, about one third or one fourth the size of a red cell. One side of the ring is thicker than the other, the chromatin being generally placed on the thinner side of the ring.

The ring—the young trophozoite, as it is called—becomes bigger, living mainly on the hæmoglobin of the cell. In order to avail the whole amount of the hæmoglobin of the red cell, it acquires amœboid movement, by means of which it projects pseudopodia on all sides. As the age of the parasite advances, the amœboid movement increases leading to the great many distorted forms when seen under microscope in a stained film.

In the meantime, the hæmoglobin which has been taken up by the parasite becomes converted into a pigment derived from the metabolism of the parasite, and is known as the *hæmozoin* particles. This pigment is fine yellow-brown or black, isolated or aggregated in clumps, and is evenly distributed throughout the cytoplasm.

The hæmozoin is insoluble in strong acids, altered by potash, but is rapidly soluble by ammonium sulphide. It is derived from the splitting up of hæmoglobin into hæmatin which, again, is derived from it and a proteid globin.

At the same time, other changes take place in the red cell. The cell becomes pale and enlarged with the presence of minute chromophilic particles stained pink with Leishman stain and known as the *Schuffner's dots.* These dote were suposed to be due to the degenerative changes of the hæmoglobin, but now it is believed to be due to cracklings on the surface of the red corpuscles due to the attachment and subsequent withdrawal of the pseudopodia of the parasite. *So far the chromatin remains single and undivided.*

When the parasite is mature, division of chromatin takes place—*Stage of Schizont* (*Schizos—spliting*)—into 2, 4, 8, 12, or more chromatin masses or nuclei. In a mature schizont, these chromatin particles vary from 15 to 20 in number.

The cytoplasm now shows signs of division and begins to aggregate round each chromatin particle, forming the so-called rosette appearance. This generally takes about 48 hours to develop from the earliest ring-form. During this period, the cell becomes very much distended with the parasite and ruptures, liberating into the circulation so many minute segments of the parasites, which contain no hæmozoin. Each of them becomes a separate and independent individual, and is known as the *merozoite.* The merozoites, which escape phagocytosis, again infect fresh red cells and the cycle is again repeated.

Thus there are three stages of the parasite in human blood which complete the cycle every 48 hours. They are

Trophozoites—Schizonts—Merozoites—Trophozoites

This cycle is known as the *human cycle or asexual cycle or cycle of schizogony.*

After the rupture of the red cell the following are set free in the circulation:—(*i*) Merozoites. (*ii*) Hæmozoin particles. (*iii*) Remnant of hæmoglobin. (*iv*) Remnants of red blood corpuscles (*v*) Toxins liberated by the parasites which have got the property of producing fever (thermogenetic) and destruction of blood corpuscle (hæmolytic).

The fate of the above are as follows:—

1. *Merozoites.*—As soon as the red corpuscles containing the parasites burst, the merozoites are set free in the circulation. Large numbers of them are washed away in the spleen. Some are devoured by the large mononuclears and macrophages and are destroyed. Some authorities doubt whether the parasites are at all engulfed. Those which escape such destruction enter into fresh red cells and complete the cycle again. In course of time, say after one or two weeks of fever, the patient becomes resistant to the parasites

which, in their turn, prepare themselves for the altered resistance and become the resistive sexual forms or *gametocytes.*

Gametocytes.—These originate from the early ring forms. They pass the trophozoite stage as described. When they become mature, instead of proceeding to the stage of schizont, they remain more or less globular, with no tendency for the division of chromatin and show very little amœboid movement. They can be differentiated into two sexes—female and male. The female or the *macrogametocyte* is bigger and more numerous than the male, containing deep blue cytoplasm when stained and a great amount of granular hæmozoin and a large mass of chromatin are placed on one side of the parasite. The male one or the *microgametocyte* is smaller and fewer in number, with pale cytoplasm, lighter pigment and a band-form mass of chromatin is placed across the parasite like a belt.

These gametocytes are the parasites which develop further in the mosquito. Formerly it was believed that these gametocytes remained in the circulation for an indefinite period, even for years. It is now believed that they live only about a week unless they are taken up by the mosquito. Muehlens has proved that inoculation of gametocytes alone into another human being is incapable of producing malaria. Manson-Bahr is of opinion that plasmodium vivax is capable of maintaining itself (after a single infection) in the human body for a period of about three years, after which it dies out, while the quartan parasite may live for 6 or 7 years, and the subtertian parasite from one month to one year.

Fate of Multiple Infection of One Red Blood Corpuscle.—More than one merozoite may enter a red cell, resulting in a multiple infection of the corpuscle. Multiple infection of the corpuscle is more common in the case of the malignant tertian or plasmodium falciparum infection.

If two parasites enter one red cell, both of them will grow into the trophozoite stage and will ultimately develop into either schizont or gametocyte. As schizonts are more frequent than the gametocytes, and of the gametocytes, again, the female one is commoner than the male, there is the possibility of the two trophozoites developing in a single red cell in one of the following combinations. According to their frequency:—

(*i*) Both of them schizonts.

(*ii*) One schizont and the other a female gametocyte,

(*iii*) One schizont and the other a male gametocyte.
(*iv*) Both of them female gametocytes.
(*v*) One female and the other a male gametocyte and lastly,
(*vi*) Both of them male gametocytes.

Thus, the stages of the developmental cycle of the benign tertian parasite are as follows:—

Trophozoites—Schizonts—Merozoites—Trophozoites

Trophozoites—Gametocytes—Mosquito—Trophozoites

2. *Hæmozoin Particles.*—These are invariably taken up by the macrophages which keep in their cytoplasm all the pigment until they are dead. The pigment is thus carried to all the organs, notably the spleen and the liver which, in their turn, become pigmented. Under the microscope the presence of the pigment in the intercellular vascular spaces are seen enclosed inside the macrophages.

3. *Remnant of Hæmoglobin.*—This is partly excreted through the urine and partly utilised by the liver in the formation of bile. If the amount of hæmoglobin is considerable, consequent upon the destruction of a large number of corpuscles, anæmia is produced. There may be urobilin in the urine or hæmoglobinuria or production of jaundice due to absorption of bile so formed.

4. *Remnant of Red Blood Corpuscle.*—These are known as shadow cells, and are cast off. The remains of the red blood cells, merozoites or the waste products of the parasites, when liberated into the circulation after the rupture of the red cell, act exactly like an intravenous injection of foreign protein resulting in protein shock. This is manifested by rigor and other symptoms.

5. *Toxin.*—That "toxins" are produced by the malarial parasites, has led to a great deal of controversy. Many authorities believe that there is no toxin, the paroxysms being produced as a result of hæmoclastic shock (protein shock), while others are of opinion that there are two different forms of toxin:—

(*a*) *Thermogenetic or pyrogenetic toxin* producing fever with rigor. This takes place simultaneously, with the bursting of corpuscles, and hence shows definite periodicity—in case of B. T. occurring every 48 hours.

(*b*) *Hæmolytic toxin* leads to the hæmolysis of the healthy corpuscles and thus contribute mainly to the production of anæmia, jaundic and hæmoglobinuria as mentioned in the last paragraph.

6. *Periodicity of the Fever.*—The development and liberation of the merozoites take place in the asexual cycle every 48 hours in the case of tertain parasites and every 72 hours in the case of quartan parasites. Two sets of tertian or three sets of quartan parasites may be introduced into the same individual by infected mosquitoes on successive days and so paroxysmal attacks, with daily paroxysms, may occur. To produce symptoms, the parasites must be numerous and so they must take 6 to 12 days from the bite of the mosquito to multiply in sufficient number.

The Mosquito Cycle

The female anopheles sucks up the human blood containing both the asexual forms and the gametocytes. As soon as the blood enters the stomach of the mosquito and becomes stationary, the asexual forms die off and the development of the malarial parasites commences from the gametocytes. It can be grouped as follows:—

FIRST STAGE, or *Stage of Maturation.*—The gametocytes, both male and female, become mature and lie motionless in the mass of blood.

SECOND STAGE or *Stage of Fertilisation.*—The microgametocyte or male parasite shows division of the chromatin into 2, 4 and 8 portions. These arrange towards the periphery of the cell, from which the cytoplasmic filamentous masses with the chromatin particles are detached from the surface and swim freely in the fluid content of the stomach of the mosquito. The filamentous mass is known as the *microgamete*. This swims in search of the *macrogamete* or the female element, probably by the process of chemotaxis, until it reaches one. The female gametocyte does not project flagella. The microgamete

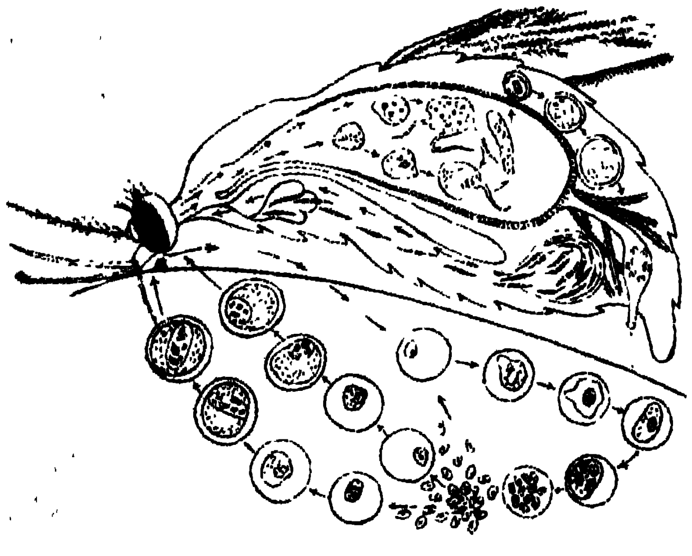


Fig. 77. Malaria parasites showing human and mosquito cycles.

enters into the macrogamete, resulting in the union of the male and female elements, with the formation of a *zygote* or *synkaryon.* This takes place inside the lumen of the stomach. This zygote becomes elongated and moves in a worm-like manner until it pierces through the wall of the stomach, and ultimately comes out and forms a cyst-like projection towards the cœlomic cavity, remaining all the while attached to the wall. The parasite, while travelling through the stomach wall, is often termed *ookinete* (moving egg) and the cyst formed by the parasite is called *oocyst.* The ookinete develops generally 12 to 24 hours after the feed of the mosquito, and oocyst takes 3 to 4 days to develop.

THIRD STAGE, or *the Formation of Sporozoites.*—The oocyst grows rapidly in size and the nucleus divides into several segments, round each of which a portion of the cytoplasm is collected, with formation of what is known as *sporoblasts.* The nucleus of each sporoblast divides into a large number of daughter nuclei, round which, the cytoplasm is collected with the formation of hundreds of elongated spindle-shaped bodies, each containing a minute chromatin. These elongated bodies are called *sporozoites.*

FOURTH STAGE, or the *Infection of the Salivary Glands.*—The cyst becomes very much distended with the large number of sporozoites until it bursts liberating the sporozoites into the cœlomic cavity. These free sporozoites then migrate to different parts of the mosquito and enter every tissue including the salivary glands, but not the ovaries. The three-lobed salivary glands usually take about 10 to 12 days to become packed up with the sporozoites. When the mosquito, with the sporozoites in the salivary glands, bites a healthy man, the parasites are liberated through the puncture into the circulation. The sporozoites enter red blood cells and become trophozoites and continue the human cycle. They can be demonstrated in the peripheral blood 10 to 12 days after the mosquito bite.

The first two stages of the mosquito cycle, *i.e.*, the maturation and the fertilisation, can be observed under the microscope outside the mosquito by examining a sample of blood containing the gametocytes. The process is accelerated by a little moisture and by breathing on the slide. Oxygen is necessary for the "ex-flagellation" to take place. Ross thinks that 250 million parasites are required to cause the paroxysm of fever. The incubation period depends on the period taken by the sporozoites introduced by the mosquito to multiply to the above number. This generally takes about 14 days.

Common Malaria Transmitting Mosquitoes are:—

Anopheles culicifacies. A. listoni. A. stephensi. A. fuliginosus.

A. rossi, the common Bengal anopheles, is readily infected in the laboratory but never found infected in nature.

Experimental Inoculation of Malaria in G. P. I.—Collect about 300 adult female A. maculipennis from stables or elsewhere in a cage. Put the cage in the incubator at a temperature of 23°C for 24 to 48 hours, to have the blood already present in the stomach digested quickly. Feed these mosquitoes daily for 5 days on a patient with numerous fully mature male and female gametocytes in the peripheral blood, through glass jars covered with mosquito netting, about 20 mosquitoes being placed in each jar. Put them in the incubator at 23°C after feeding, keeping the air of the incubator thoroughly moist with wet clothes. Give occasional feed to keep them alive. Glucose solution on cotton wool may be substituted for human blood as food for the mosquitoes. Allow them to bite the patient to be inoculated artificially 10 to 15 days after the first infectious feed.

PLASMODIUM MALARIÆ (Parasite of Quartan Malarial Fever)

(*Laveran* 1881)

Life History.—Same as P. vivax except the following:—

Red blood corpuscle not enlarged. There are no Schuffner's dots. Pigment is abundant. This is the most pigmented parasite. Amœboid movement is less. The equatorial-belt form is characteristic. The pigmented cytoplasm is often placed like an equatorial belt across the middle of the infected red corpuscle. Rosette appearance characteristic. Merozoites are 6 to 12 in number. Cycle of schizogony 72 hours. Gametocytes are rare.

PLASMODIUM FALCIPARUM (Parasite of Malignant Tertian Malaria)

(*Also called Laverania malariæ*)

Life History.—Same as P. vivax except the following:—

1. All the points mentioned below in differentiation between Plasmodium and Laverania.
2. Ring forms are smaller than the benign tertian, and consist practically of the dots of chromatin with very little cytoplasm; they are often placed on the margin of the corpuscles, and are called accole forms (*accole—on* shoulder).

3. Fragmentation of the chromatin into two in ring forms.
4. No change of red blood corpuscles.
5. Presence of Maurer's clefts—coarser than Schuffner's dots. Forming irregular cleft-like markings on red blood corpuscles.

Some authorities like to group this under the genus Laverania from the presence of the following differences:—

	Plasmodium.	*Laverania.*
Schizogony	in peripheral blood	in internal organs (bone marrow, spleen, brain, liver etc.).
Schizonts ..	numerous in peripheral blood.	absent in peripheral blood.
Cycle of schizogony ..	regularly in 48 hours.	irregular (more or less continuous).
Gametocytes ..	like schizonts	crescents.
Tendency ..	benign	malignant (by blocking the capillaries of vital structures, *e.g.*, brain due to the special tendency of the organism to clump together to form masses.)
Multiple infection of corpuscles ..	rare	common.

Morphology of the Crescents.—The crescents are the gametocytes of the Laverania malariæ and appear 7 to 10 days after the commencement of the attack, the life-span being 30 to 60 days. They are quinine-resistant and show no amœboid movement. They are of both sexes—male and female. The females are larger than the males. Thus macrogametocytes are female crescents, microgametocytes are male crescents. The male crescents, like the gametocytes of other malarial parasites, produce ' flagellated bodies,' in the stomach of the mosquito. These are not found in the female gametocytes.

Size.—10-15μin length and 2-5 μ in breadth. Occupies nearly the whole of the red corpuscle leaving the remainder on the concave side of the crescent.

Female Crescent (*Macrogametocyte*).—Larger and thinner than the male. Hæmozoin pigment clumped together in the centre, chromatin rounded and placed in the centre. The female crescents are more numerous than males. The protoplasm is finely granular.

Male Crescent (*Microgametocyte*).—Smaller and thicker than the female. Hæmozoin spread out occupying the middle two-thirds of the body. The protoplasm is hyaline.

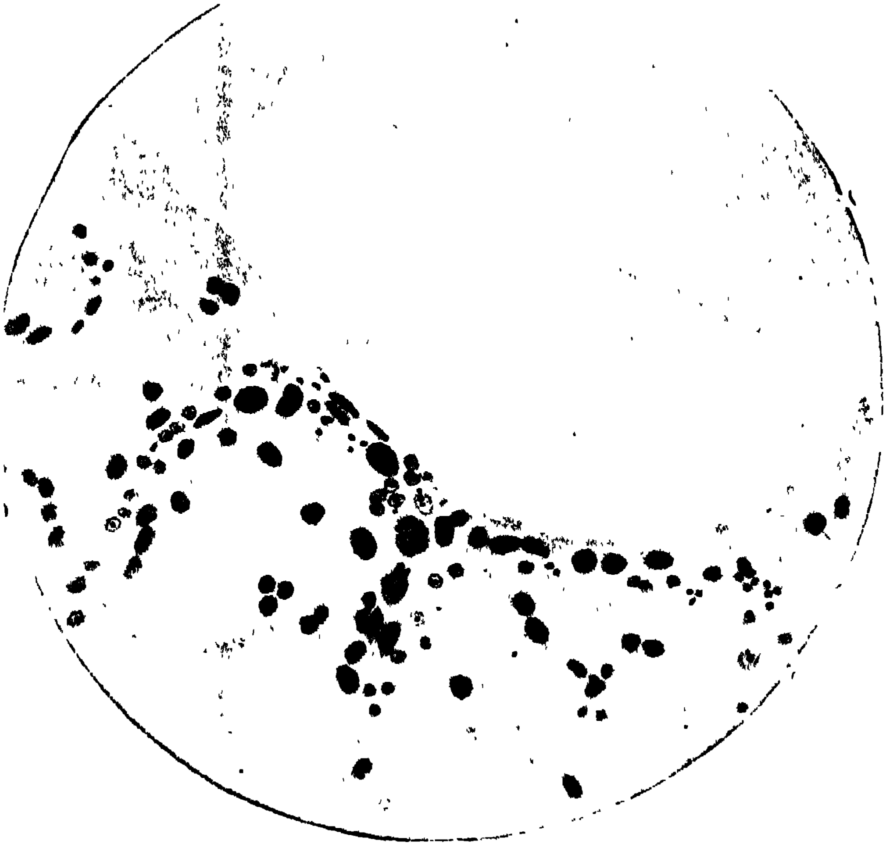


Fig. 78. Subtertian malaria parasites in the capillaries of brain. (*Dr Gopal Ch. Chatterjee*).

Chromatin.—Elongated and occupies more space.

Mixed Infection.—Mixed infection, with more than one species or even all the three species, both asexual and the gametocyte forms, is fairly common (*cf.* the coloured plate).

Spontaneous Cure and Immunity

Spontaneous Cure.—Let us take a case of benign tertian infection after the bite of a mosquito. Supposing 10 sporozoites are introduced. If nothing prevents their multiplication, these will multiply to 10×15 or 150 parasites after 48 hours, 150×15 or 2,250 on the 5th day and so on, until on the 15th day these will count 1,371 million organisms, or five times the minimum dose according to Ross. Thus, fever commences on the 15th day after the mosquito bite. If sporozoites proceed to multiply at this rate, it will take a few days more —about a week or so—to infect all the red cells of the human blood. Moreover, the hæmolytic toxin has already destroyed a large number of uninfected corpuscles.

Thus, we find that a man will rarely survive more than a month or so if he remains untreated. But this never happens in nature. On the other hand, spontaneous cure—although temporary—is the rule. This indicates that enormous numbers of the parasites are constantly being destroyed in the system. This usually takes place in the spleen. The parasites, on the other hand, to protect themselves from extinction become resistant-forms or gametocytes and remain in the deeper organs until taken out by the bite of the mosquito.

It is not uncommon to find gametocytes in cases of malignant tertian—the crescents—in the blood of a man who is apparently in good health and not suffering from malaria.

Relapses.—Relapses are more commonly associated with the parasite of the benign tertian malaria. It has been found that there is a febrile threshold depending on the total number of the malarial parasites in the blood stream of the patient. This number has been generally estimated to be one thousand million. Parasites below this number will not be able to produce fever.

In untreated or improperly treated cases the spontaneous resistance offered by the body to destroy the parasites is such that the number of parasites falls below the above febrile threshold and so the patient remains afebrile. Chill or other factors will lower the resistance, so that the schizogony cycle flares up, the number of parasites rises above the febrile threshold and a relapse occurs.

Immunity.—There is no real immunity. The so-called immunity is more or less a tolerance only. It is doubtful whether protozoal diseases ever produce any immunity. It is a noticable fact that children of the endemic areas often run about fever-free and in robust health, although with a large number of parasites in blood and a huge enlargement of spleen, while a foreigner suffers very frequently and much more severely. It has been observed during the therapeutic inoculation in cases of G. P. I. that many patients become wholly immune afterwards. Infact, it has been reported by Manson-Bahr that in one instance " it was impossible to convey malarial infection, either by mosquito bite or by direct blood inoculation, to an individual who had lived many years in India and also who had suffered much from malaria in his youth."

Cultivation of the Malarial Parasite.—This was first done by Baas and Johns who described a culture medium in which the parasites grow readily. Row has invented a process of cultivating the parasites from the finger blood. *The technique is as follows:*—

Draw 10 c.cm. of blood from the patient's vein and put in to 0.1 c.cm. of a 50% sterile dextrose solution without forming air bubbles. Defibrinate the blood with sterile wire. Put the blood in narrow test tubes so that a column of blood plasma deep forms above the settled blood corpuscles. Incubate at 40°C. Malarial parasites grow at the topmost layers of blood corpuscles just below the clear plasma layer. During subculture the leucocytes must be removed as they devour the newly emerged merozoites.

Pathology of Malaria

Blood.—As the malarial parasites live on the hæmoglobin of the human red blood cells, it is evident that some form of anæmia must set in. This is further enhanced by hæmolysis of the red cells not actually attacked by the parasites. It has been estimated that there must be at least one parasite to every 100,000 red blood cells in order to produce symptoms, while in a very heavy infection we often come across cases where nearly every fifth red cell is attacked by a parasite.

Apart from the anæmia, the hæmoglobin index is also reduced to 50 or 30 per cent or even less. Degenerative changes of R. B. C., *e.g.*, poikilocytosis, basophilic stippling, anisocytosis, etc., are also present in advanced cases. Leucocytes may increase during the paroxysms

DESCRIPTION OF PLATE

MALARIA PARASITES IN PERIPHERAL BLOOD

Fig. 1.—All stages of Benign tertian malaria parasites.

Fig. 2.—All stages of Sub-tertian malaria parasites.

Fig. 3.—All stages of Quartan malaria parasites.

Fig. 4.—Showing multiple infections of all forms of malaria parasites and microfilaria.

From a case admitted into the Medical College Hospital on the 29th November 1903 for fever and jaundice. Every field was full of all the forms of parasites—from 10 to 12 crescents being present in a single field along with fully developed benign tertian and quartan forms. Reported by Dr. Gopal Ch. Chatterji in *The Lancet* (Aug. 25, 1906, P. 499.)

Plate VI

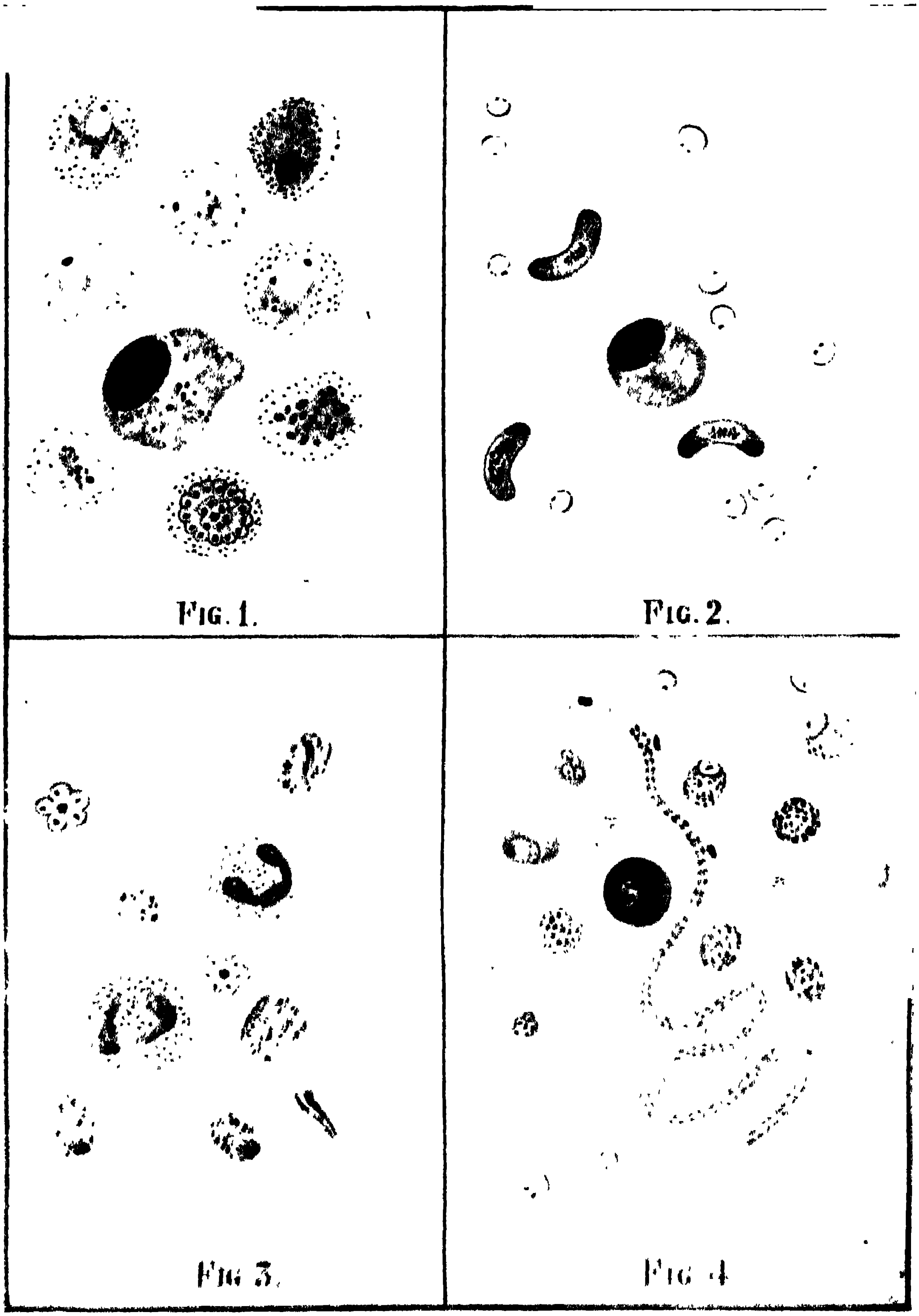


Fig. 1. Fig. 2. Fig. 3. Fig. 4.

Malaria Parasites in peripheral blood

Face Page 226

but are usually diminished with a ratio of 1 : 900 R. B. C. (normal being 1: 625), but showing an increase of large mononuclears which often show presence of ingested hæmozoin in them. Blood sugar is diminished.

Spleen.—This is enlarged and pigmented. In acute cases the capsule is greatly stretched. On section, the pulp is soft, dark coloured and intensely congested. Hæmozoin and hæmosiderin are found in considerable amounts.

Liver.—Enlarged, congested, soft and pigmented. Flow of bile is excessive due to hæmoglobinæmia and is responsible for bilious symptoms.

Bone marrow, kidneys, and *other organs* show similar changes.

Brain in a fatal case of subtertian infection shows congestion of vessels of the pia mater. The cortex is pigmented while the white matter shows punctiform hæmorrhages due to blockage of the capillaries by the parasites.

Pathology of Black Water Fever. It mainly resembles the pathology of sub-tertian malaria. The kidneys are enlarged, congested, pigmented, capsule strips off easily. Histologically, tubules appear blocked by hæmoglobin infarcts, hyaline or blood casts. Hæmozoin pigment is also present. Blood picture is indicative of severe anæmia with evidence of regenerative changes. Black water fever is now generally believed to be due to continuous infection or repeated attacks of subtertian malaria.

Inoculation.—Human blood containing malarial parasites on inoculation into lower animals always give negative results. Inoculation into man is always successful.

Malaria Infection in Fœtus.—Parasites have been found in the blood of child, both before birth and when seven days old. Infection takes place through the maternal blood, through tears of placenta into the fœtal circulation. Fœtal death takes place due to intense infection of the placenta.

Laboratory Diagnosis of Malaria

First examine, whether the film is properly drawn and stained or not. In an imperfectly stained specimen it is very difficult to identify the parasite, often impossible. Hence it will be a waste of time and energy to proceed to examine a badly made and badly stained slide for malarial parasites. The slide should be rejected or stained once more.

How to know whether the slide is properly stained? Search for a lymphocyte or a large mononuclear cell. Observe whether the cell shows distinctly a blue cytoplasm and pink or brownish-red nucleus. If they are distinct and sharply defined then the staining is good.

Method of restaining a slide stained with Leishman's stain. Remove the cedar wood oil from the slide, if there be any, by xylol, allow it to dry thoroughly. Wash with absolute alcohol or rectified spirit till no blue colour comes out. The film may remain pink still. Dry in air and stain with Leishman's stain as usual.

Points to note in order to identify malarial parasites.

1. Never give an opinion unless you see both red and blue in the parasites, chromatin being red and cytoplasm blue.
2. The parasite is always inside a red corpuscle.
3. Examine that portion of the slide where the corpuscles are isolated and distinct.
4. Examine with the oil immersion lens with the condenser up.
5. Familiarise yourself with the vacuoles in red blood corpuscles which are often mistaken for malaria parasites. Vacuoles are sharply defined, do not take any stain and contain no pigment. Malarial parasites are not sharply defined; they take the proper stain and contain pigment.
6. Don't mistake a blood platelet on a red blood corpuscle as a malarial parasite. The platelet is surrounded by a clear zone and never by a blue stained body.
7. Enquire whether the patient had quinine recently as the parasites disappear after quinine.
8. Don't give a negative opinion unless searching carefully for at least 15 minutes. More than one slide may have to be examined.
9. Give your report "No malarial parasite seen" and not "No malarial parasite present."

Usually one species is found in a blood smear but more than one species may be present in the same slide in all the stages

Forms of the parasites found in a smear.

1. *Ring form or trophozoite stage.*—One, rarely more, small oval rings inside a red cell, size of the ring being about 1|5th the diameter of red blood corpuscle (diam. of R. B. C. normally is about 7μ). The ring may be irregular showing amœboid movement of the parasite. One pole of the ring (*i.e.*, opposite the red dot—the chromatin) is distinctly thicker than the other, the whole of the ring being stained blue and representing the body of the parasite. The chromatin is placed at one end and is stained bright red. There may be a few pinkish dots—the Schuffner's dots—outside the ring but inside the corpuscle (this is only found in B. T. infection and not in other species).

2. *Adult form or schizogony stage.*—The parasite is much bigger than the ring form, occupying the major portion of the red cell, which again, in its turn is swollen up (only in B.T.). The margin of the parasite, the body of which is stained blue, is irregular, indicating the amœboid movement of the parasite.

Morphology of the Malaria Parasites

	Benign Tertian.	Quartan.	Malignant Tertian.
Trophozoite	Young trophozoite large. Pseudopodia long with much amœboid movement	Smaller than B. T.; Larger than M. T. Pseudopodia not marked.	Smallest, generally near the edge of R. B. C., multiple infection common. Accole forms characteristic.
Schizont			
Size	Larger than red blood-Corpuscles, (more than 7μ).	Smaller than red blood Corpuscles (less than 7μ).	$\frac{1}{2}$-$\frac{2}{3}$rd red blood corpuscles, much smaller.
Shape	Irregular.	Regular, equatorial band-characteristic.	Irregular
Movement	Active amœboid movement.	Sluggish in immature forms.	Active.
Pigment	Diffuse. (often invisible)	Coarse and granular, peripherally arranged.	Fine, scanty.
Segmenting form	Rosette form	Characteristic rosette	Irregular.
No. of segments	15-20	6-12	10-20 or more.
Infected corpuscles	Pale and enlarged with Schuffner's dots in deeply stained specimens.	Not enlarged; no Schuffner's dots.	Not enlarged. Corpuscle distorted with Maurer's dots (rare).
Gametocyte	Resemble growing trophozoites	Resemble trophozoites	Crescent.
Duration of schizogony	48 hours	72 hours	48 hours or less.
Distribution of parasites in the peripheral blood.	Large numbers in peripheral blood. Shows all phases.	About equal numbers in peripheral and visceral circulation. Shows all-phases.	Scanty compared with the enormous numbers in the visceral blood. Shows only rings and crescents.

Two forms of granules are found, one inside the parasite—dirty brown in appearance—the hæmozoin particles, and the other outside the parasite but inside the red cell—light-pink in colour—the Schuffner's dots (only in B.T.). The chromatin is divided into two or more according to the age of the parasite, arranged in order to form a rosette when mature. In the quartan type the parasite is often found placed transversely across the red cell—so called Equatorial band.

3. *Sporulating forms or merozoite stage.*—The parasite divides into 15 to 20 round blue small ones which are liberated into the plasma after bursting of the red cell. Each merozoite consists of a blue cytoplasm and a red chromatin. This stage is difficult to demonstrate in a smear preparation. It is believed that several hundreds of sporulating parasites are necessary to be capable of producing the symptoms.

4. *Gametocyte form or sexual stage.*—Looks like a developed trophozoite with a thick blue mass with undivided chromatin. It is more or less round showing little or no amœboid movement. The sex can be differentiated as follows:—

Male.—Chromatin large, slightly elongated and placed centrally.

Female.—Larger than the male, chromatin large, round and placed on one side. In sub-tertian form.—The gametocytes are crescentic in appearance and hence known as crescents.

The life of gametocytes is much longer than the schizonts. Benign tertian and quartan gametocytes take about 5 days to reach maturity and the crescents about 10 days.

Extrusion of flagella-like processes appear from the male gametocytes during examination under cover glass. This naturally takes place in the stomach of mosquito.

Thick blood film.

This is often preferable, specially when the parasites are few. A big drop of blood is placed in the centre of the slide and then spread with the corner of another slide, over an area of about ½ inch in diameter. It is, then, thoroughly dried and then dehemoglobinized by keeping the slide in distilled water or in a mixture of 2 per cent. formalin with 1 per cent. of glacial acetic acid. After all the hæmoglobin has been extracted, the slide is rinsed thoroughly in water, dried and stained as usual.

Liquid blood film.

This is mostly used to examine the parasites in their living state, unstained so as to demonstrate the amœboid movement of the parasites so distinct in the early stages.

Students are always accustomed to see the leucocytes in a stained specimen and so do not realise that the leucocytes are colourless. In a liquid blood-film the leucocytes can be seen in their natural state and their amœboid movements, the necessary factor in phagocytosis, can be well demonstrated.

Method of preparation of the liquid blood film.

Prick the finger, squeeze out a small drop of blood, touch the drop of blood with the middle portion of a slide and gently put a cover glass on. Avoid any pressure on the cover glass and see that these is no air bubble under the cover glass. In order to avoid drying of the blood surround the cover glass with a thin layer of vaseline. Examine under the high power of the lens and finally examine under oil immersion lens.

HÆMOPROTEUS (HALTERIDIUM)

These are found in the nucleated red corpuscles of birds. Nearly every Calcutta pigeon harbours these parasites. It is transmitted from one pigeon to the other through the bite of the hippoboscid fly.

PIROPLASMA

These are found in the red blood corpuscles of warm-blooded mammals, especially dogs. In man they cause Oroya fever of South America, though this has not yet been definitely confirmed. It is transmitted by a tic.

HÆMOGREGARINA

These are found in both the cold-blooded as well as the warm-blooded animals. In the cold-blooded animals, they live in the nucleated red blood corpuscles. In the warm-blooded animals, they live in the mononuclear leucocytes.

COCCIDIUM

This generally affects the lower animals. Man is also affected. This is generally found in the bile passages, liver, intestines or body tissue. The common human parasites are (1) Isospora hominis and (2) Eimeria wenyoni.

Life History.—Almost identical with plasmodium (the malarial parasite); the oocysts are passed with the fæces, and develop into sporozoites. Man is infected by the swallowing of the oocysts when the sporozoites are liberated which enter the cells of the biliary passages as well as of the intestinal mucosa.

Pathogenicity.—In the liver they produce papilliform areas resembling adenocarcinoma. The coccidia are seen in the cells as well as free in the cavities.

RHINOSPORIDIUM KINEALI

This parasite was found in the nasal tumour from a case of O'Kineali, in 1894. Ashworth maintains that rhinosporidium infection was first described by Malbran in 1892 and also that the nomenclature Rhinosporidium seeberi (Wernicke 1903) is prior to that of R. kineali named by Minchin and Fantham 1905. Further, the splendid monograph on this affection by Ashworth has undoubtedly proved that the organism is not a protozoon but a fungus. The growth consisted of a granulation tissue and numerous cystic areas containing the parasite. Since Kineali's observation a fairly large number of rhinosporidium infection has been reported by workers all over India.

The vegetative phase is unknown. Only the cystic phase is known which contains the sporozoites. When the cyst ruptures the sporozoites are liberated. The mode of transmission is not known.

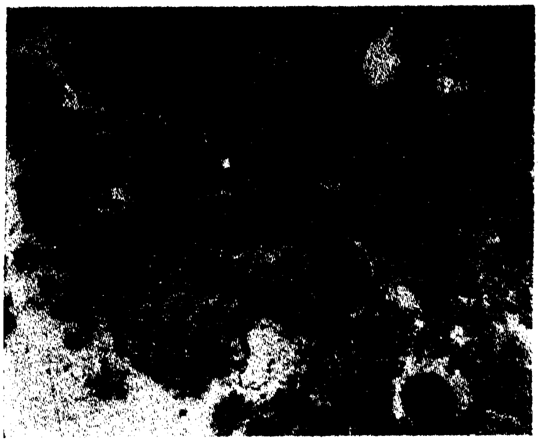


Fig. 79. Rhinosporidum kineali in section of tumour.

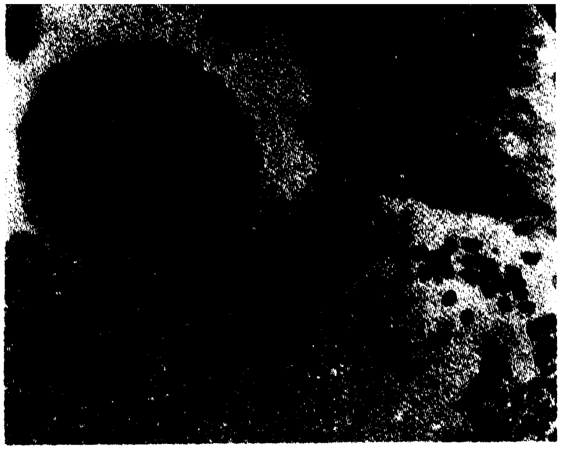


Fig. 80. Same as Fig. 79 under high power.

SARCOSPORIDIA

As the name implies the organism lives in muscles. Man is rarely infected The infection occurs through eating half-cooked infected meat.

CILIOPHORA

There is only one organism belonging to this class which is of human importance.. This is *Balantidium coli* or *Paramœcium coli.* It is a highly complex organism and nearly all the functions of a fully developed mammal are carried out by this unicellular organism. It is fairly big, measuring 50-70 in length. It is surrounded on all sides by short cilia by means of which it moves freely with rapid darting motions The mouth or the cytostome is a circular opening with a funnel-shaped peristome. The cytoplasm contains a nucleus, two contractile vacuoles and numerous food particles, including red blood cells, leucocytes, starch, fæcal matter, etc. They live in the cæcum and may also be found in the appendix.

Reproduction takes place by binary transverse fission. *Pathogenicity.*—Usually harmless, but may cause dysentery, which nearly always ends fatally.

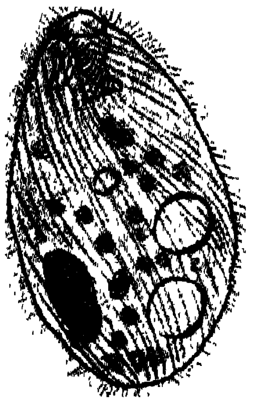


Fig. 81. Balantidium coli.

CHAPTER XVII

HELMINTHES OR WORMS

Helminthes or worms are metazoal organisms.

Parasitic worms are divided into two phyla as follows:—

- (1) Platyhelminthes or flat worms.
    - (*a*) Cestodes or tape-worms—alimentary canal absent.
    - (*b*) Trematodes or flukes—alimentary canal present.
- (2) Nemathelminthes or round worms.

(1) Phylum Platyhelminthes

CLASS.	FAMILY	GENUS.	SPECIES.
Cestoda (*alimentary canal absent*).	Tæniadæ	Tænia.	T. saginata.
			T. solium.
			T. echinococcus
		Hymenolepis.	H. nana.
	Dibothriocephalidæ.	Dibothriocephalus.	D. latus.
Trematoda (*alimentary canal present*).	Fasciolidæ.	Fasciola.	F. hepaticum.
		Dicrocœlium	D. lanceolatum.
		Clonorchis.	C. sinensis.
		Opisthorchis.	O. felineus.
			O. noverca.
		Paragonimus.	P. westermanii.
		Fasciolopsis.	F. buski.
		Heterophyes.	H. heterophyes.
	Paramphistomidæ.	Gastrodiscus.	G. hominis.
	Schistosomidæ.	Schistosomum.	S. hæmatobium.
			S. mansoni
			S. japonicum.

(a) Cestodes or Tapeworms

General Characters.

Size varies from ¼ inch to several feet in length. They are flattened, tape-like and elongated, hence the term tape-worm.

Head.—Small, contains 4 and rarely 2 suckers, with or without hooklets.

Body.—Segmented, each segment being called a ***proglottid,*** which is in itself a complete individual, and contains a branched uterus, ovaries, testes, etc. The last segments are mature and are full of eggs.

Genital System.—These are hermaphrodites, each segment containing both the male and the female sexual organs. Male sexual organs consist of testes, vas deferens and penis; female organs consist of uterus, ovaries, oviduct, seminal receptacle, vagina and yolk gland.

Alimentary Tract.—Absent.

Life History.

The adult or mature stage (also called ***strobila***) inhabits the alimentary tract of the host, through the fæces of which the eggs of the worm (*onchospheres*) leave the host.

The next stage is spent outside the host until it is taken up by the intermediate host through food. The egg-shell is digested in the alimentary tract of the intermediate host, liberating the contained organism which is then called a *scolex.* The scolex pierces through the wall of the alimentary tract and migrates to distant parts through blood vessels or otherwise and remains encysted, this stage being called *cysticercus.* When the living embryos are taken with improperly-cooked meat, they enter into the alimentary tract of the definitive host and become the adult worm.

The terms used to denote different stages of the worm are:—

Strobila—The adult worm. *Proglottides.*—Segments. *Rostellum.*—Beak-like projection from the head in the definitive host, usually man or carnivorus animals. *Onchosphere.*—Eggs. *Scolex.*—The embryo in its resting stage (only the head). *Cysticercus.*—The cyst containing the scolex in the intermediate host, usually a herbivorus animal.

TÆNIA SAGINATA

(*Syn.—T. mediocancellata or Beef Tape-Worm*)

Geographical Distribution.

Widely distributed in the tropics. It is found in the beef-eating population, hence unknown amongst the Hindus. The Mahomedans and the Europeans get infected.

Morphology.

Strobila.—Fairly large, white worm measuring 12 to 25 feet in length.

Head.—Small, visible to the naked eye, slightly flattened and contains 4 suckers. There is no rostellum or hooklet.

Neck.—Short.

Segments or Proglottides.—Numerous, anterior segments are small, immature and are broader while the posterior segments are large, mature and longer.

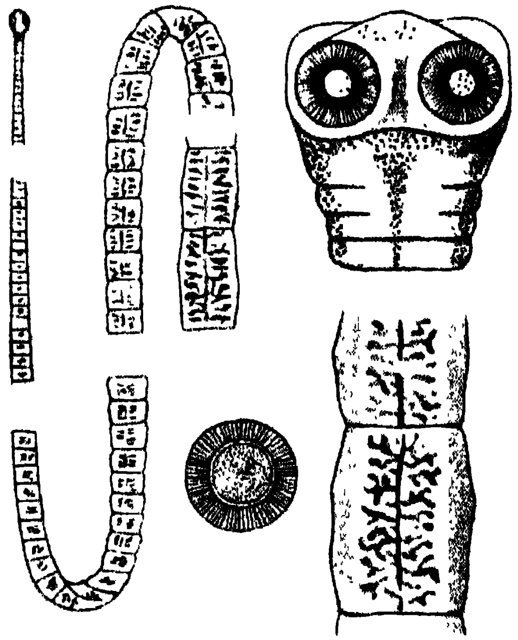


Fig. 82. Tænia saginata.

Genital Openings.—These are situated at the side of each proglottides and are placed usually on alternate sides of the segment.

Genital Organs.—Uterus is branched and placed in the middle line of the segment, forming a prominent canal-like structure; hence the term medio-canellata (median canal).

Eggs.—Are circular, consisting of a thick double shell with large number of radial striations. Inside the shell, the space is full of minute granules with 4-6 hooklets in them.

Life History.

The adult worm lives in the intestine (usually the small intestine) of man. The mature proglottides separate off from the strobila and rupture either before or after the exit from the intestines and discharge the eggs contained therein. The eggs come out of the fæces and contaminate the grass from which the cattle is infected during grazing. The egg shell is dissolved in the stomach of the animal with liberation of the scolex which migrates into the muscles and other structures and forms a cyst-like structure called cysticercus. It often penetrates the mucous membrane of the mouth and pharynx and passes to the muscles of mastication where cysticercus is formed. When the meat of this animal is taken by another human being and if the scolex is not destroyed through imperfect cooking or during mastication, it gets into the intestine and grows into an adult form. If the eggs are swallowed by man instead of the cattle, he gets the scolex and then can act as the intermediate host.

Pathogenesis.

Man is the definitive host. The parasites are the least harmful. Slight anæmia, digestive disturbances and occasional reflex irritability may occur. When man becomes the intermediate host, single or numerous cysticercus affections occur in various parts of the body, notably liver, brain, eye, etc. (cysticercus cellulosa), which may be fatal, if vital centres are affected.

TÆNIA SOLIUM

(*Syn.—Pork Tape-worm*)

Geographical Distribution.

Rarely found in India, found in European countries, especially in Germany.

Morphology.

Strobila.—Smaller than Tænia saginata, but of the same appearance and colour, measuring 6 to 10 feet in length.

Head.—*Small,* rounded, contains 4 lateral suckers. There is a rostellum with 30 to 40 hooklets attached to it, arranged usually in double rows.

Segments.—These resemble Tænia saginata. They are numerous, anterior segments are small, immature, and broader than long, and the posterior ones which are large, mature and longer than broad.

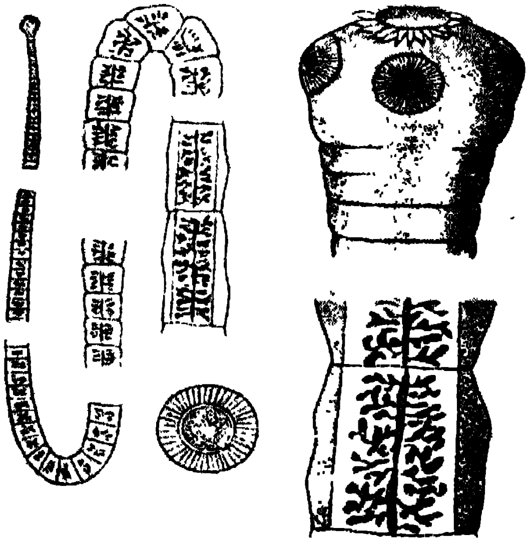


Fig. 83. Tænia solium.

Genital Pores.—Same as Tænia saginata and are usually arranged in alternate segments.

Genital organs.—Uterus is often branched.

Eggs.—Yellow and round resembling those of Tænia saginata.

Life History.

Strobila lives in human intestine, scolex in the muscles of pig. If the eggs are swallowed by man scolex will be present in his muscles, liver, brain, eyes, lungs, etc., as cysticercus cellulosæ, and may remain for years.

Pathogenesis.

Man is the definitive host. The worms cause anæmia and gastro-enteritis. Man as intermediate host—cysticercus occurs in the tissue with local symptoms.

TÆNIA ECHINOCOCCUS

(*Dog Tape-worm*)

Adult forms are found in dogs, etc., Man is the intermediate host.

Geographical Distribution.

In those places where man has got a close relation with dog, especially in Australia, South America.

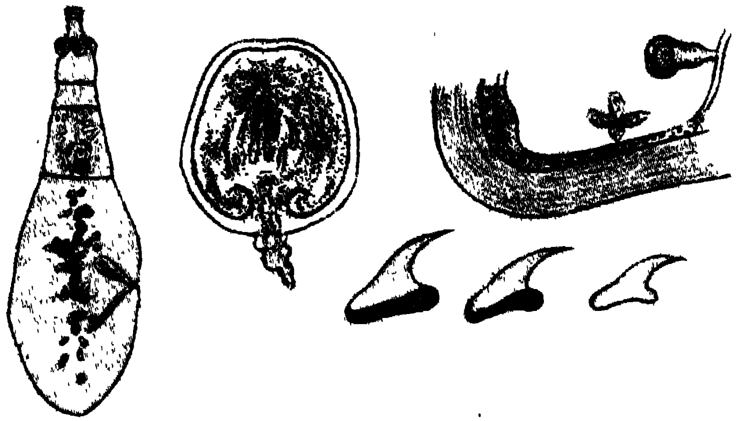


Fig. 84. Tænia echinococcus.

Morphology.

Strobila.—The smallest tape-worm known; ¼ inch long.

Head.—Very small, round, contains 4 lateral suckers, and a rostellum with 14 to 20 hooklets.

Segments.—There are altogether three segments only, the last one being sexually mature.

Genital Pores.—Placed laterally, and usually arranged alternately.

Eggs.—Thin-shelled.

Life History.

Adult form is found in the intestine of dogs. Eggs come out in dog's fæces. When they are swallowed by man, the shell is dissolved, setting free the scolex which migrates to the different parts of the human body, specially the liver (57%), lungs (8%), kidneys, brain, heart, genital organs, spleen, etc., and remains encysted as the *Hydatid Cyst.*

Pathogenesis.

It causes the hydatid cyst in man.

The Hydatid Cyst

It takes fairly long time to grow, and several months pass before it is of some size.

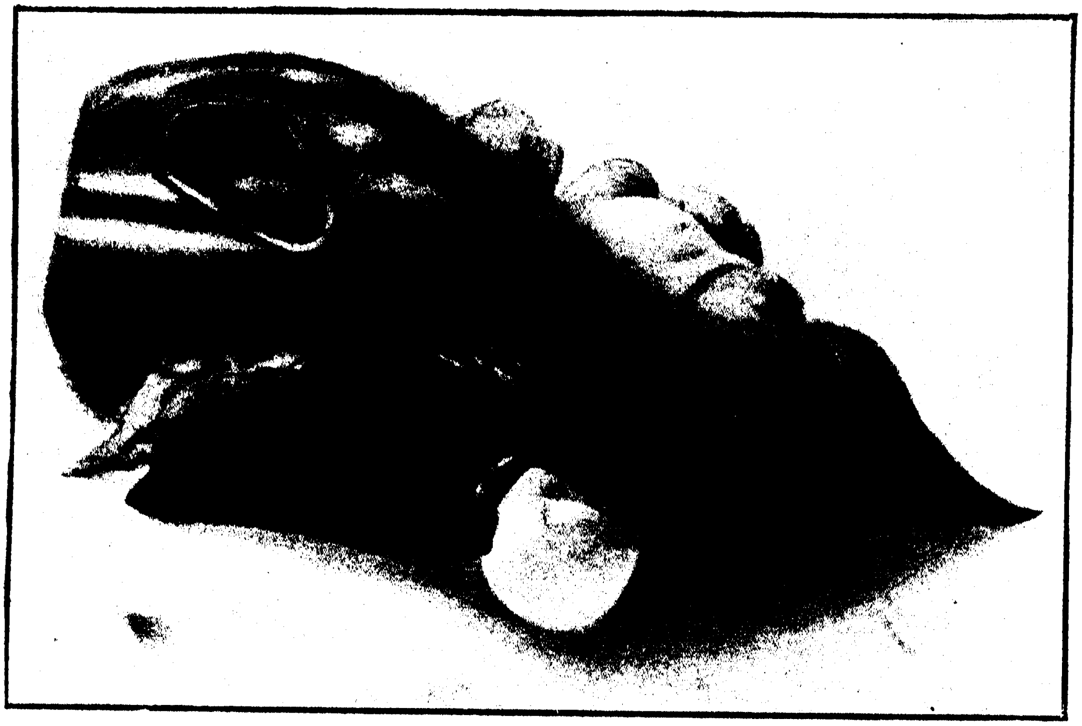


Fig. 85. Multiple Hydatid cysts of liver. (Dr. Ganapati Panja)

Cyst Wall.—Thick, brittle, rolls up when broken and consists of two layers:—(*a*) Outer or ectocyst—cuticular, laminated, dense (*b*) Inner or endocyst—granular, cellular and is the germinating layer. *Contents.*—(1) Clear or slightly opalescent fluid, neutral in reaction, specific gravity 1005-1008. It contains no albumen except after repeated tappings. There is a trace of chloride and a Fehling-reducing substance, succinic acid. (2) Scolex with numerous hooklets.

Formation of the Cysticercus:- -

(1) The cyst may be single or multiple and of different size.

(2) As the cyst grows, the parasite multiplies, with formation of new cysts from the inner parenchymatous layer. Thus, daughter cysts—" brood-capsules "—are produced inside the mother cyst.

(3) Occasionally, on account of the presence of some defect in the cuticular layer, the daughter cysts, along with the parenchymatous layer, escape through the outer layer and grow outside the mother cyst in the tissue.

Multilocular Cyst.—Large number of independent cysts held together and surrounded by dense fibrous tissue.

Sterile Cyst.—When the cyst does not contain any scolex (indicated by the absence of hooklets).

DIBOTHRIOCEPHALUS LATUS

(*Syn. Fish Tape-worm*)

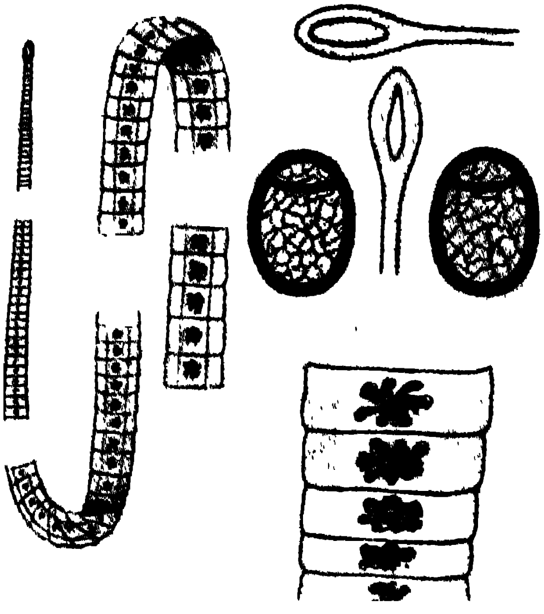


Fig. 86. Dibothriocephalus latus.

Geographical Distribution

Common in Europe, America, etc.

Morphology.

Strobila.—Largest tapeworm—15 to 30 feet long.

Head.—Elongated, with two lateral elongated grooves or suckers; hence the term dibothriocephalus (*di* two, *bothrios* suckers. *cephalos* head). There is no rostellum, no hooklet.

Segments.—It possesses largest number of segments. All the segments, both anterior as well as the posterior, are broader than long.

Genital Pore.—Placed centrally and posteriorly.

Genital Organs.—Uterus large and coiled, not branched.

Eggs.—Large, granular and possess a hinged lid at the top.

Life History.

Strobila lives in man, scolex in a kind of fish known as pike. If the eggs are swallowed by man they develop into strobila. Thus man always remains the definitive host.

Pathogenesis.

Anæmia of a very severe form.

TABLE SHOWING THE CHARACTERS OF THE COMMON CESTODA OF MAN

Name.	Length	Head.	Segments.	General Appearance.	Ova.	Habitat.	Intermediate Host.	Source of infection.
Tænia solium.	6-10 ft.	4 suckers, rostellum, 30-40 hooklets.	700-1000 mature ones longer than broad.	Uterus-a central canal with few branches. Opening—on the side of segment, alternately.	Spherical	Human intestine	Pig.	Improperly cooked pork.
Tænia saginata.	12-25 ft.	4 suckers, on rostellum, no hooklets.	1000-2000 same as T. solium.	Uterus-a central canal with numerous branches. Opening—same as T. solium.	Spherical	Human intestine.	cattle.	Improperly cooked beef.
Tænia echinococcus.	¼ inch.	4 suckers, rostellum, 14-20 hooklets.	3 only, last segment is mature.	Uterus-cavity-like and in the last segment which alone is mature. Opening—on the side.	Spherical	Intestine of dog.	Man.	Food contaminated with fæces of dog containing ova.
Dibothriocephalus.	15-30 ft.	2 suckers, club-shaped, no rostellum, no hooklets.	3000-4000, all segments broader than long.	Uterus coil like. Opening—in centre.	Oval, with a hinged lid.	Human intestine.	Fish (pike)	Improperly cooked infected fish.

HYMENOLEPIS NANA

(*Syn.—T. nana or Dwarf tape-worm*)

Specially found in children.

Morphology.

Strobila.—Very small worm measuring about ½ inch in length. The head is black and the body white.

Head.—Four suckers, a rostellum which can be protruded and retracted and a single row of hooklets.

Segments.—Small and broader than long.

Genital Pore.—Marginal.

Genital Organs.—Uterus uninterrupted.

Eggs.—Pale; shell contains no structure.

Life History.

Strobila lives in human intestine; intermediate host not definitely known, probably an insect, or snail, or none.

Pathogenicity.

Nil.

TREMATODES

[*Syn.—Flukes, Distoma (di-double, stoma-mouth)*]

General Characters.—These are flattened unsegmented elliptical organisms. They get their nourishment from the host, on whom they fix themselves by means of suckers or spines. They are hermaphrodites except the bilharzia which are bisexual and, unlike cestodes, possess an alimentary tract which ends blindly without any excretory opening. The adult worms are found in the vertebrate host and the larval ones in some form of small snails. All of them possess two suckers hence called *distoma.*

Flukes Divided according to their Site of Occurrence.

1. *Liver Flukes.*—Fasciola hepaticum, Fasciola lanceolatum. Clonorchis sinensis, Clonorchis endemicus.
2. *Lung Fluke.*—Paragonimius westermanii.
3. *Intestinal Flukes.*—Gastrodiscus hominis, Fasciolopsis buski, Heterophyes heterophyes.
4. *Blood Flukes.*—Schistosomum hæmatobium, Schistosomum mansoni, Schistosomum japonicum.

LIVER FLUKES

1. Fasciola hepatica.

Situation.—Occurs in the bile passages of sheep, and rarely of man. In sheep it causes a very fatal affection called *liver-rot.*

Morphology.—It is flat and elliptical, measuring ½″ to 1″ in length and ¼″ to ½″ in breadth.

Head.—Small and projects from the body, and contains one sucker anteriorly.

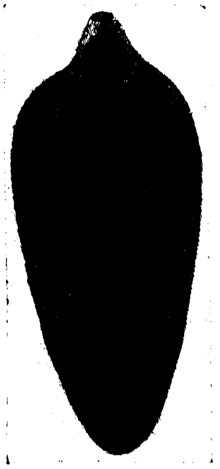


Fig. 87. Fasciola hepatica.

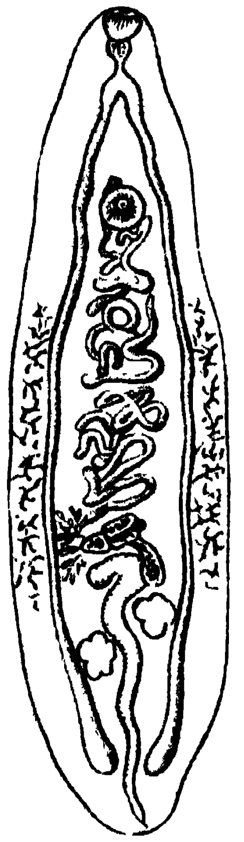


Fig. 88. F. lanceolata.

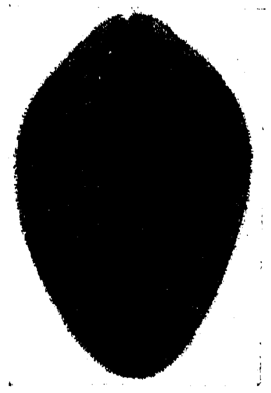


Fig. 89. P. westermanii.

Body.—There is another sucker at the most anterior end near the one on the head.

Genital Openings.—Placed between the two suckers.

Genital apparatus.—It is hermaphrodite, the whole of the body being composed of the uterus and the testes. MALE ORGANS.—These consist of a series of coiled up testes, vas deference and vasa efferentia. FEMALE ORGANS.—Large coiled up tube-like uterus, ovary, oviduct and branched yolk gland.

Eggs.—Oval, with one end slightly flattened, forming a lid-like structure.

Life History.—The eggs come out with the fæces and contaminate water in which the embryos are liberated through the lid.

The embryo is a ciliated organism, and is known as the *miracidium.* It swims freely in the water until it enters into a fresh-water snail (Limnœus minutus), which acts as the intermediate host. The embryo then develops into a sporocyst in the tissue of the snail, mainly in the liver; and, when fully developed, they become elongated and possess a sac like alimentary canal and are known as *rediæ*. The sporocyst ruptures and the redia comes out of the snail and swims in the water again, this stage being called the *cercaria*.

It enters through the mucous membrane of the definite host (man or sheep) while drinking the contaminated water and migrate towards the liver.

Pathogenicity.—It is usually non-pathogenic, but may produce inflammation or obstruction of the bile passages.

2. **Fasciola lanceolata.**—(Syn.—Dicrocœlium lanceolatum or Distomum lanceolatum). Resembles Fasciola hepaticum except—

It is more slender and smaller in size. It is of lancet shape with the anterior end more pointed. The two suckers are widely separated.

3. **Clonorchis sinensis.**—Syn.—Opisthorchis sinensis.

This fluke is common in Japan and China, and is the commonest human parasite. It is elongated, flat, transparent and measures about ¼ to ½ inch in length. The infection is caused by eating uncooked fish.

Intermediate Host.—There are two different intermediate hosts—a primary one, *viz.*, mollusc, and a secondary one, *viz.*, fish.

It inhabits the bile ducts.

4. **Clonorchis endemicus.**—Similar to Clonorchis sinensis.

5. **Opisthorchis feleneus.**

Usually found in the bile passages of the cat, man being rarely infected.

Distribution.—Siberia.

Intermediate host.—Fish.

6. **Opisthorchis noverca.**

Affects man and often found in India.

LUNG FLUKE

Paragoniums westermanii

Distribution.—China, Japan, Phillippines, etc.

Morphology.—The worm measures about ⅓ inch long and is more or less globular and reddish-brown in colour. Transverse section is circular and longitudinal section oval. The ventral surface is slightly flattened. General arrangement of the suckers, genital apparatus and genital pores are similar to the Fasciola hepaticum.

Eggs.—Numerous and are expectorated from the lung through the sputum. They measure about 1 mm. long and 0·5 mm. broad.

Life History.—Not definitely known. The first intermediate host is a fresh-water snail (*melania*) and the second intermediate host is probably a fresh water crab.

Pathogenicity.—Man is infected by eating raw or improperly cooked crabs. They cause hæmoptysis and live in the bronchi and burrow in the lungs. They may also infect liver, brain and intestines.

INTESTINAL FLUKES

1. **Fasciolopsis buski.**

Fairly common in India, inhabits small intestines.

2. **Gastrodiscus hominis.**—Syn.—Amphistomum hominis.

Distribution.—India and other tropical countries.

Morphology.—Small worm measuring ¼″ long, ends thick, posterior part disc-shaped and anterior part tapering. There are two suckers.

Eggs.—Very small—150 μ ×70 μ

Pathogenicity.—Occasionally dysenteric symptoms.

3. **Heterophyes heterophyes.**—Syn.—Distomum heterophyes.

Very small fluke—smallest known, with ventrally situated and separate acetabular and genital suckers, 2 mm. in length and 1 mm. or less in breadth; cuticle with rectangular scale and with numerous glands on the anterior ventral surface. Found in the human subject.

BIOOD FLUKES

(*Schistosomum hæmatobium—Schistosomum mansoni—Schistosomum japonicum.*)

S. hæmatobium.—(Syn.—Bilharzia hæmatobia, Distomum hæmatobium).

It inhabits the portal capillaries and the vesical veins.

Distribution.—Africa, Japan, China, West Indies and other tropical countries.

Morphology.—Unlike other flukes, the worm is unisexual, the male and female being separate individuals.

The male worm is larger, thicker and stouter of the two. It is flat or leaf-like and measures about ½ inch in length. The cylindrical appearance is due to infolding of the sides. The surface is rough, due to numerous fine spines. The female is longer, thinner, round and possesses a smooth surface. Both of them have two suckers at the anterior extremity, between which the sexual aperture opens.

They live separately until they mature, when the male worm forms a canal by infolding the sides of the flat body (known as the gynæcophoric canal) assuming a more or less rounded appearance.

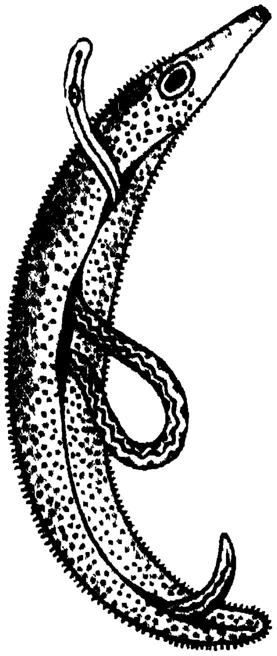


Fig. 90. S. hæmatobium.

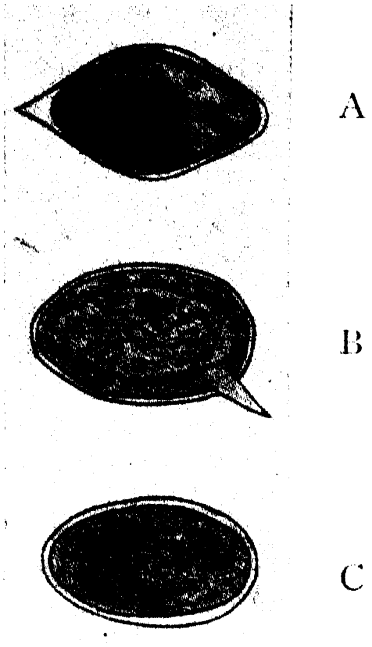


Fig. 91. Eggs of Bilharzia
A.—Egg of S. hæmatobium
B.—Egg of S. mansoni
C.—Egg of S. japonicum.

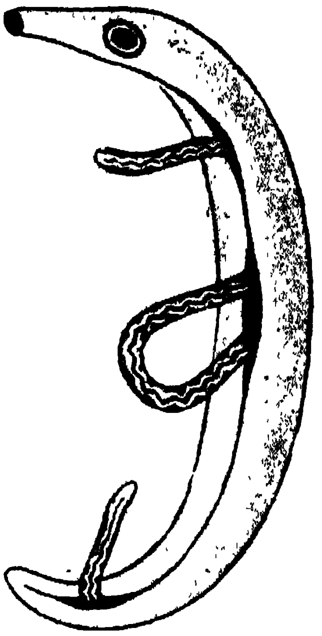


Fig. 92. S. japonicum.

In this form, it circulates through the portal vein, until a mature female enters into the gynæcophoric canal meant for her and remains in this position for fecundation with the sexual openings in apposition. The pair then travels against the blood current until the further passage is obstructed. The female leaves the male and deposits the eggs.

Ova.—Numerous, often block the portal capillaries. They measure about $160\mu \times 60\mu$ and are oval, finely granular and possess a thin shell. The chief peculiarity is the presence of a spine projecting from one end (terminal spined). Parts of the future embryo can be clearly made out inside the shell.

Life History.—The eggs are voided with the urine and contaminate the water in which a ciliated actively motile ***miracidium*** is developed. The miracidium enters a snail (Bullinus), and develops into a ***cercaria*** in the liver, and comes out of the snail to live in the water in order to enter the human host through the skin or to be taken with drink. It takes about 6 weeks to complete its cycle in the snail. The snail discharges the cercaria, which process continues for weeks. The cercaria swims in water and enters into the unbroken human skin while the man is bathing or washing in or drinking the contaminated water.

Pathogenicity.—Schistosomiasis or endemic hæmaturia of Egypt.

Schistosomum mansoni

Adults.—Same as Schistosomum hæmatobium, only slightly smaller.

Inhabits.—Rectal capillaries of the portal vein.

Ova.—Same as Schistosomum hæmatobium except that the spine is placed on the side (lateral-spined ova).

Intermediate Host.—The snail Planorbis boissyi.

Pathogenicity.—Rectal bilharziasis (symptoms resemble dysentery and bleeding piles.)

Schistosomum japonicum

Adult.—Same as Schistosomum hæmatobium except the following:—(1) There is no spine on the surface of the male. (2) Inhabits hepatic capillaries of the portal vein.

Ova.—Same as Schistomum hæmatobium except:—

(1) There is no spine. (2) It is much smaller.

Intermediate Host.—The snail Blanfordia nosophora.

Pathogenicity.—Katayama disease, characterised by fever, enlargement of the liver and the spleen, ascites, diarrhœa and cachexia.

(2) Phylum Nemathelminthes (of Metazoa) Round

CLASS.	FAMILY.	GENUS.	SPECIES.
Nematoda.	1. Ascaridæ	Ascaris	A. lumbricoides RW
	2. Oxyuridæ	Oxyuris	O. vermicularis TW
	3. Strongylidæ	Ankylostoma	A. duodenale. HW
		Necator	N. americanus.
	4. Trichinellidæ	Trichurina	T. trichiuria.
		Trichinella	T. spiralis.
	5. Filariidæ	Filaria	F. bancrofti.
			F. loa.
			F. perstans.
			F. demarquayi.
			F. ozardi.
	6. Dracunculidæ.	Dracunculus	D. medinensis.
	7. Angiostomidæ.	Strongyloides	S. stercoralis.

General Characters.—Filiform non-segmented, bisexual worms with an alimentary tract, inhabiting the alimentary tract and tissues. Females are larger than the males.

ASCARIS LUMBRICOIDES

(*Syn.—Round Worm*)

Distribution.—Cosmopolitan, and is the common worm of the intestines, especially in the children.

Morphology.—The worm is elongated, not divided into segments like the tape worm and is bisexual. The female measures about 8-12 inches in length. It is pinkish-white in colour when fresh from intestine, gradually changing to white.

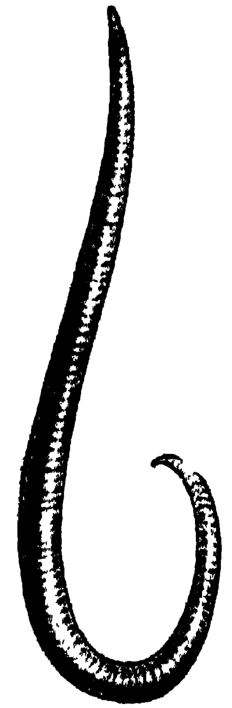


Fig. 93. Ascaris lumbricoides

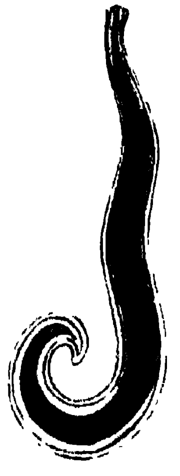


Fig. 94 & 95. Oxyuris vermicularis— male and female.

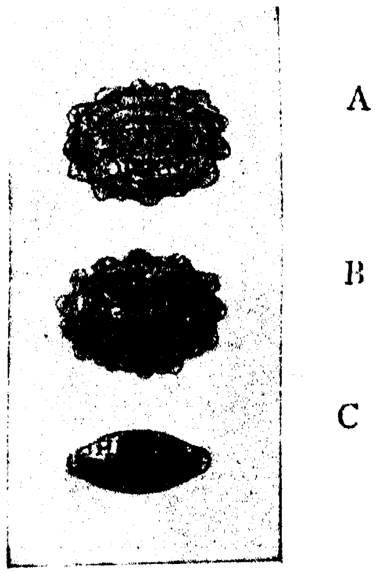


Fig. 96. A and B—Egg of ascaris. C—Egg of T. dispar.

There are four longitudinal ridges running through the whole length of the worm with numerous transverse striations all through.

Mouth parts consist of three papillæ or lips round the oval aperture, one dorsal and two ventral.

Alimentary tract extends from one end of the worm to the other. Sexual aperture is placed at the junction of the anterior and the middle thirds. Sexual organs occupy the posterior half of the body.

Posterior end of the male is curved and has several pairs of postanal papillæ with two large lance-like spicules.

Eggs.—They are very characteristic. They are oval, bile-stained, measure about 0·05 mm. in length and are surrounded by an irregular membrane; occasionally the membrane is missing, giving a smooth outline. The contents are granular and fill up the whole egg when unfertilised. In a fertilised egg, the ovum does not fill up the whole egg shell, and is finely granular.

Life History.—The eggs are voided with fæces and, in fresh stool, are found in an unsegmented condition. It takes a fairly long time to develop into an embryo, depending mainly on temperature and moisture. The embryo remains coiled up inside the egg shell, and remains so for years, until it is swallowed by another human host, when the egg shell is dissolved; the embryo being set free passes to the small intestine where it lives.

Stewart is of opinion that the eggs hatch out in the intestines, and the embryo migrates to the lungs through the liver, where the development stages are passed. From the lungs they pass to the trachea, pharynx, œsophagus and the intestines.

Pathogenicity.—Usually non-pathogenic, but may cause—

(*a*) *Mechanical symptoms* mainly due to migration and obstruction of the different channels, *e.g.*

1. Pass into the stomach and the œsophagus when they are vomited out.
2. Obstruct the bile duct, or, the pancreatic duct, or even, pass on to the gall bladder.
3. Pass through gastroduodenal or typhoid ulcers of the intestines, leading to perforation, etc.
4. Obstruction of the eustachian tube has been recorded.
5. Pass to the larynx, pharynx, etc.
6. Castellani reports a case of acute appendicitis caused by an attempt of an ascaris to force its way through the appendix.
7. May produce volvulus by aggregation into masses or even intestinal obstruction.

(*b*) *Toxic.*—Nervous irritability—convulsion, vertigo, itching of nose or anus, etc.

(*c*) Ill-defined gastric and nervous symptoms, *e.g.*, dyspepsia, nausea, foul breath, peevishness, abdominal pains, etc.

OXYURIS VERMICULARIS

(*Syn.—Thread Worm, Pin Worm or Scat Worm*)

Very common in children, inhabiting the large intestine, especially the rectum.

Morphology.—This is a very small worm, the female measuring about ½ inch long, and the male ⅓ inch. The posterior end of the male is slightly curved and blunt, that of the female elongated and tapering.

Genital Opening—is placed in the upper third.

Eggs.—Small, thin-shelled, oval, one side being convex and the other plane, not stained with bile, containing a coiled-up embryo which is often visible inside the egg.

Life History.—The eggs are swallowed by children, usually through their contaminated finger-nails (auto-infection). The egg shell is dissolved, and the adults develop in the small intestine, where the union between the males and females takes place. After sexual union the male dies. The female goes to the large intestine, where she becomes mature and generally lodges in the rectum, giving birth to a large number of eggs.

Pathogenicity.—Usually non-pathogenic, except the local irritation caused by the adult females round the anus, causing inflammation which often extends to the vulva and vagina in female children.

ANKYLOSTOMA DUODENALE

(*Syn.—Hook-worm*)

Distribution.—All tropical and subtropical countries.

Habitat.—The adult worm lives in the small intestine of man. particularly in the jejunum, less often in the duodenum.

Morphology.—The female is larger and straighter than the male, but not in the same proportion as is found in other nematodes, the proportion being one male to three female worms. The female measures 10-13 mm. in length and the male 8-10 mm.

They are elongated, cylindrical, generally of white colour when fresh, changing to grey when dead. The anterior end is the narrowest and the posterior the broadest part of the worm, and especially so in the male.

Mouth Parts.—Mouth parts are fairly strong, powerfully armed with four strong claw-like hooks and two conical teeth. The hooks are arranged on the ventral line, two on each side; and the teeth on the dorsal line, one on each side.

Posterior End.—The male possesses a large umbrella-like projection supported by 11 ribs. This is known as the copulatory bursa, and is used to fix the female worm during copulation. The cloaca opens at the bottom of the bursa with two long delicate spicules projecting from it.

In the female, the posterior end is blunt, conical with a short spine at the end, and possesses no copulatory bursa.

Genital Opening.—In the male, at the posterior end, and in the female, on the ventral surface at the junction of the middle and posterior third of the body. On account of the different position of the sexual openings, the worms appear like the letter Y during conjugation.

Eggs.—Very numerous, oval in shape, with rounded ends, not bile stained. The egg shell is very thin with a clear space inside containing usually four granular masses or segments. Eggs, containing less than 4 segments, are very rare in the fæces On the other hand eggs with more than four segments are fairly common, especially when the fæces is kept for some time.

Fig. 97 & [illegible]. Ankylostoma duodenale (male and female).

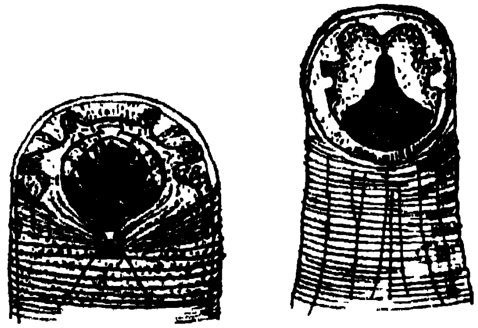


Fig. 99 & 100. Head of A. duodenale and N. americanus

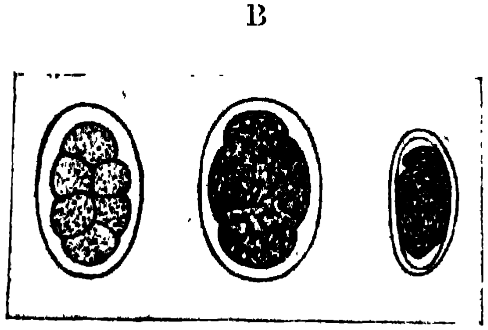


Fig. 101. A and B—Ova of Ankylostoma C—Oxyuris ova.

Life History.—When the fæces containing the eggs is kept in a suitable condition of temperature and moisture, the segmentation proceeds very rapidly from four to eight, sixteen or more, until the whole thing is converted into a granular mass, subsequently changed into a rhabditiform embryo, moving inside the egg shell. It often takes only one or two days to develop into an embryo, which untimately comes out of the egg shell and grows rapidly, being very active and devouring voraciously any organic matter present.

During a period of one week it moults twice, after which it ceases to eat and becomes quiescent, and lives in this condition in the water or damp earth for weeks or even months. Whenever they come in contact with the skin or mucous membrane of human beings, either during handling the soil, or taken with drinking water or earth (as eaten by children or geophagists), they enter into the skin (through the hair follicles) or mucous membrane, then pass to the subcutaneous or submucous tissue, blood or lymph vessels, subsequently to the right side of the heart and then reach the pulmonary circulation. Leaving the pulmonary capillaries they proceed to the air vesicles, and then to the bronchi and trachea. Finally they reach the pharynx, from which they pass into the œsophagus, stomach and, ultimately, to the small intestines.

It is believed, that the larva acquires the power of resistance against the action of gastric juice by the round-about passage, known as the indirect route. The infection through the so-called direct route, *i.e.*, by feeding, is regarded to be due to the passage of the larva through the wall of the œsophagus to the blood stream, pulmonary circulation, and so like the indirect route.

It has been suggested by Sambon, and proved by others, that some of the larvæ pass directly from the skin to the intestine through the blood stream.

Pathogenicity.—It produces a severe and progressive anæmia usually associated with dyspeptic symptoms, the affection being known as ankylostomiasis or uncinariasis. It has been estimated that, to produce the well-marked symptoms of ankylostomiasis, 500 to 1000 worms are required to be present in the intestines for a period of at least six months.

NECATOR AMERICANUS

Distribution.—Formerly it was believed to occur mainly in America, but it is now found to occur in various tropical and subtropical countries, particularly India.

Morphology.—Closely resembles Ankylostoma duodenale. It is smaller and more slender than ankylostoma. Oral capsule consists of one pair of semilunar plates and two pairs of spines. Caudal bursa of the male is divided into two.

Eggs.—Resemble those of ankylostoma but slightly bigger.

Life History.—Same as A. duodenale.

TRICHIURIS TRICHIURA

(*Syn.—Trichocephalus dispar, Whip-worm*)

Distribution and Habitat.—Common in India and Egypt, and inhabits mainly the cæcum.

Morphology.—It is small and, as the term " whip worm " implies, consists of a very thin, thread-like (the lash of the whip) anterior portion, a thick posterior portion forming the handle of the whip.

The female is larger and straighter, and the lash constitutes the anterior two-thirds. It measures 40-50 m.m. The male is smaller, the posterior end being spirally coiled.

Eggs.—Oval, thick-shelled, bile-stained, and possess two characteristic button-like processes at the two ends.

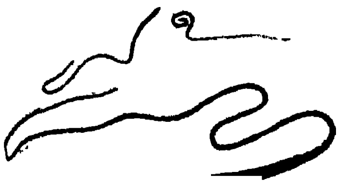


Fig. 102. T. dispar. (Natural size and enlarged)

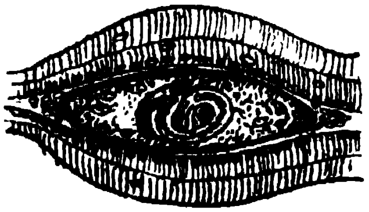


Fig. 103. Encysted larva of Trichinella spiralis in muscle.

Life History.—Eggs come out with the fæces. The embryo develops in moist soil and is taken up by human host with contaminated food.

Pathogenicity.—Usually non-pathogenic. They live in the cæcum, or in the appendix. It is believed that they feed on blood.

TRICHINA (TRICHINELLA) SPIRALIS

Distribution.—World-wide, mainly Germany.

Morphology.—A small worm just visible to the naked eye. The anterior end is tapering. Posterior end thicker and blunt, and forms the anus.

Male.—Posterior end contains two pairs of papillæ.

Female.—Genital opening opens at the anterior fifth of the body.

Life History.—Eggs are found in the upper portion of the uterus. Inside the uterus they change into embryos, which come out from the worm and migrate to the different muscles where they remain encysted. When the muscle containing these encysted forms are eaten by human beings with food, infection takes place, and the adult worm emerges in the stomach and enters the small intestines.

FILARIA BANCROFTI

(*Syn.—Microfilaria nocturna, Filaria sanguinis hominis nocturna*)

Distribution.—Found throughout the tropical and sub-tropical countries.

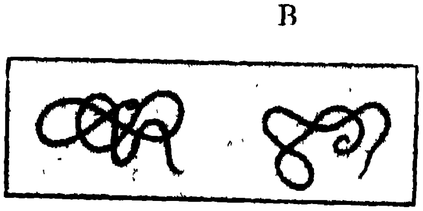


Fig. 104. Filaria bancrofti (natural size). A—female. B—male.

Morphology.—Adult male worms are rarely seen. They are long thread-like, measure about 3-4 inches in length. They inhabit big lymphatic vessels especially of the lumbar region and the thoracic duct. The female worm is larger and thicker than the male and gives birth to a series of embryos commonly known as microfilaria and may live many years.

Eggs.—Rarely found unless the mature female aborts. The embryo can be seen coiled up inside the egg.

Embryos.

These are the commonest form observed. They inhabit the circulating blood and may be as many as 500-600 in every drop.

Morphology of the Embryo.—It is a minute elongated, slender, transparent, snake-like organism surrounded by a thin capsule which is slightly bigger than the organism. The anterior extremity is rounded off, and the posterior extremity tapers to a fine point. It is 0.3 m.m. long, and the diameter is about the diameter of a red blood corpuscle.

Anterior End or the Head.—It is blunt and thick, and possesses a fine spine based on a highly refractile granule. This can be protruded very suddenly, and as rapidly withdrawn. It performs the important function of piercing the capsule or sheath while the organ is fixed in the stomach of the mosquito.

Body.—It constitutes nearly the whole of the embryo worm. It is filled up with granules except at the anterior extremity. About one-fifth from the anterior end, there is a trangular gap, called by Manson the 'V' spot. There is a similar gap at the posterior end termed by Manson the 'tail spot.' 'V' spot is the rudimentary vascular system. 'Tail' spot is the rudimentary excretory system. When stained with hæmatoxylin or Leishman stain, the granules are seen to be well stained but deficient at the 'V' spot and the 'tail' spot.

A sort of viscus, mainly composed of granular mass, is seen at the posterior part of the middle third, which is lined by a delicately striated wall. This is the rudimentary alimentary canal of the adult worm.

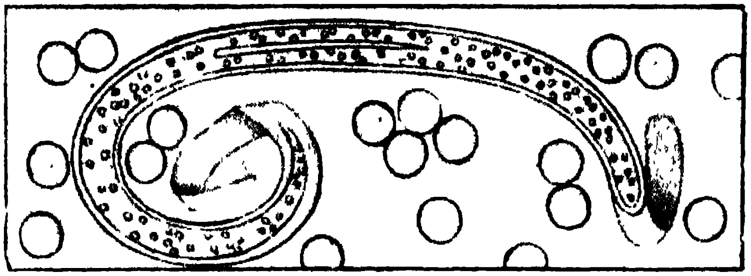


Fig. 105. Microfilaria bancrofti in blood.

Periodicity.—The microfilaria is rarely seen in the peripheral blood in the day time. They begin to appear in the peripheral blood from the evening, reach the maximum at midnight, and gradually decrease as the night advances, and practically disappear in the morning.

It has been observed that, if a filarial patient is made to sleep during the day and remain awake at night, the periodicity is reversed.

The purpose of the nocturnal periodicity is to meet the intermediate host—the mosquito *Culex fatigans* or *Aedes variegatus.*

Manson observed that during the day they usually reside in the pulmonary and large vessels. Filaria infection in the islands of the Pacific show no periodicity of the microfilaria.

Life History.—The embryos circulate in the blood, and the sheath or capsule prevents its migration away from the blood channels. The nocturnal periodicity brings the microfilaria to the peripheral circulation to be taken up by the mosquito culex, stegomyia or anopheles. The blood in the stomach of the mosquito gets clotted, and the sheath partially fixed. The embryo makes vigorous attempt to pierce the sheath by violent forward and backward movements and, with the aid of the spine situated on the head, it bores the sheath and comes out into the blood, whence it migrates to different parts of the mosquito until it reaches the thoracic muscles. Here, metamorphosis occurs, and the embryo moults thrice, during which period, the vascular, alimentary and excretory systems develop from the V-spot, viscus and tail-spot

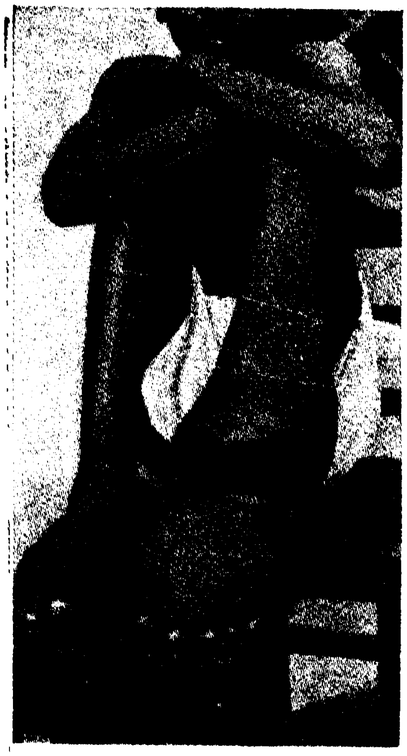


Fig. 106. Elephantiasis left foot.

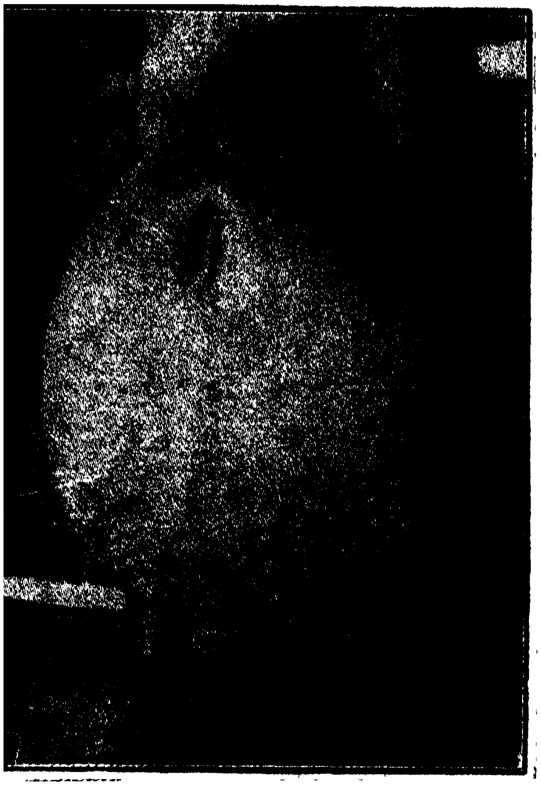


Fig. 107. Elephantiasis Scrotum.

(From Carmichæl Medical College Hospitals)

respectively. Thus, the embryo is transformed, in about 10-20 days, into a fully formed adult parasite, though immature. It then leaves the thorax and passes through the prothorax into the head of the mosquito and remains coiled up, generally in pairs at the base of the proboscis waiting for the next feed of the mosquito when it penetrates the membrane uniting the labella to the tip of the proboscis-sheath and finally enters into the human body through a puncture made in the neighbourhood of the site of the mosquito bite. From the skin it enters the lymphatic channels and lodges in the bigger lymphatic vessels and glands. Here it becomes sexually mature, the male disappears, and new generation of embyos are set free into the lymph. They then pass through the thoracic duct and finally into the circulation.

Pathogenicity.—Filarial diseases are produced by the adult filaria and never by the embryos. These are mainly caused by obstruction and irritation. It takes about 15-20 years before symptoms of obstruction appear. Irritation produces inflammation.—Lymphangitis, abscess, synovitis, orchitis, arthritis, funiculitis, etc.

Obstruction leads to dilatation of the lymph channels, producing varicose glands, lymph scrotum, lymphatic varix, elephantiasis, dropsy, chylous ascites, chylous diarrhœa, chylous pericardium, chylocele, etc.

It is a noticeable feature that, in most cases of elephantiasis, no microfilaria is found on examination of blood. This is believed to be due to either the adult filariæ (1) having died, or (2) having so obstructed the lymphatics that no microfilaria can enter the circulation.

The adult filaria are very often found calcified in the lymphatic gland. It is also believed that the presence of staphylococcus or streptococcus infection further enhances the obstruction and subsequently the fibrotic changes.

Filaria loa.—These are thicker and longer than filaria bancrofti. The microfilaria loa resembles bancrofti in all respects except in periodicity (which is diurnal), as they appear in peripheral blood during the day time and disappear at night. The intermediate host is a diurnal blood sucking fly Chrysops.

Pathogenicity.—It causes *Calabar swellings.*—These are fugitive swellings appearing suddenly and disappearing gradually in about three days, and may occur in any part of the body.

DRACUNCULUS MEDINENSIS

(*Syn.—Filaria medinensis or Guinea-worm*)

Distribution.—The Punjab, N. W. Provinces, Central India, Rajputana, and Arabia, Persia, Africa. Females generally come to the surface during the monsoon months.

Morphology.—The male is rarely seen—smaller than the female. It dies soon after copulation. The female is a long, cylindrical worm, whitish in colour and measuring about a metre in length.

Anterior end is blunt and consists of a mouth, which is bounded by two lips. In a mature adult, the whole body is filled up with the pregnant uterus.

Genital Opening.—Just behind the mouth.

Embryos.—Are flattened, coiled up with a tapering tail.

Life History.—The adult worm is found in the human connective tissues, mainly near about the ankles, where a small blister is found and through which the uterus of the worm is prolapsed and hundreds of embryos are liberated. This takes place only when the part is in contact with water. The embryos are set free in the water and enter into a crustacea *cyclops*, the intermediate host—inside which further development takes place. Infection takes place when water containing the cyclops with the guinea-worm larvæ is swallowed. The cyclops is digested and the parasite, set free in the stomach, burrows into the tissues and migrates to different places.

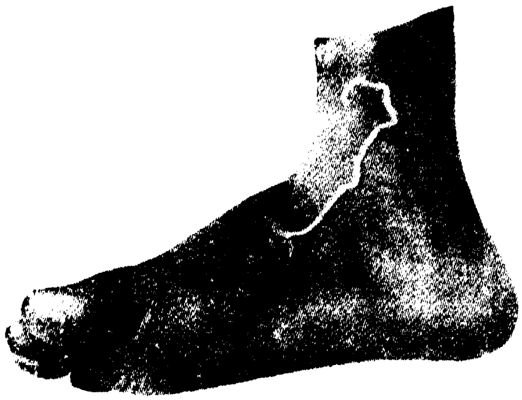


Fig. 108. Female guinea worm in process of extraction. (Dr. N. B Mandal's patient)

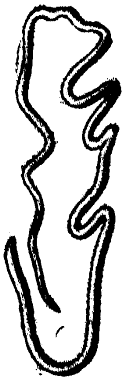


Fig. 109. Dracunculus medinensis (greatly reduced).

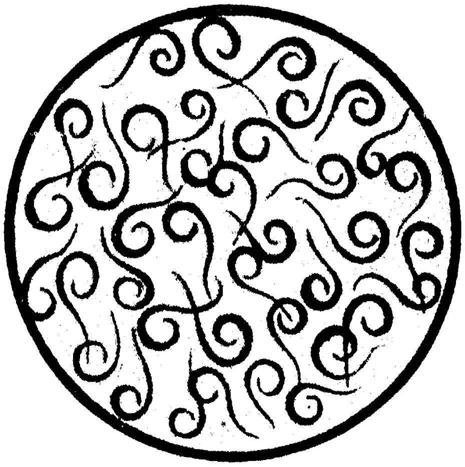


Fig. 110. Embryo of guinea-worm.

Pathogenicity.—The female worm generally travels from one part of the body to another through the subcutaneous tissue until she reaches the ankle. Here a blister is formed which ruptures, liberating the embryos when it comes in touch with water. The whole worm gradually comes out through this opening, specially when cold water compress is applied over it and the worm wound round a matchstick. No attempt should be made to pull the worm out, otherwise it will rupture and suppuration will result.

CHAPTER XVIII

ARTHROPODA

The arthropoda, as the term implies, are animals with jointed legs. During recent years, they have attracted the serious attention of scientists on account of the transmission of some of the deadly diseases by many members of this phylum. They are of much importance for their injurious activities to human beings by destroying crops, daily necessities of life, and even, health itself. We are concerned here with the last of their activities. They are a menace to human beings, in that they contaminate food, transmit disease from the sick to the healthy, and do themselves cause certain diseases.

General Characters of the Arthropoda

Arachnoidea (tics and mites).

Body consists of the three regions—Pro, meso, and metasoma, often all fused together with no such differentiation as head, thorax and abdomen. Exoskeleton consists of chitin only.

Appendages—No antennæ, mouth parts consist of a pair of four-jointed pedipalps (palps) ensheathing the piercing apparatus, known as the cheliceræ. There are four pairs of walking legs, only three pairs being present in the larval stage.

The **Sarcoptidæ** (the *mites*) are eyeless. The male dies after copulation while the female burrows in the skin commonly between fingers, on the flexor surface of the forearm etc., and is lodged at the blind end of the burrowed tunnel, depositing eggs along its tract. The female passes through four stages, (1) the larva, (2) the nymph, possessing no sex organs, (3) the pubescent female and (4) the adult female bearing eggs. The human itch mites are Sarcoptes scabiei (causing scabies) and Demodex folliculorum (hair follicle mite).

The **Ixodidæ** (the *tics*) possess four pairs of legs, two pairs of mouth parts and no antennæ. The *head* consists of the piercing parts, hypostome or the dart, and a pair of piercing chitinous substances, the cheliceræ. Between these piercing parts lies the mouth (See figures 59 and 60). Ixodidæ are divided into the following groups.—

Sub-family	Group and Species.
Argasinæ	—Argas, (A. persicus, transmitting fowl spirillosis); Ornithodorus (O. moubata, transmitting tic fever, O. savigni).
Ixodinæ	—Dermacentor (D. andersoni transmitting spotted fever of the Rockey mountains); Ixodes (I. ricinus); Hyalomma (H. ægyptium); Margaropus (M. annulatus transmitting Texas fever which is caused by Babasia bigemina, a protozoon); Amblyomma (A. hebræum).

Insecta.

Body divided into head, thorax, and abdomen. *Appendages.*—Head bears one pair of antennæ and mouth-parts consisting of one pair of palpi and a proboscis, intended for piercing and sucking or sucking only. Throax bears three pairs of walking legs. Some of them possess no wings. Abdomen bears no appendage.

During metamorphosis, the eggs are succeeded by worm-like larvæ which are extremely voracious at this stage. These are then followed by the pupa stage in which they remain encased quiescent and do not feed. The next stage is the imago or the fully developed insect.

The most important orders of this class are:—

Diptera (Mosquitoes and flies, sandfly, simulium, midges, etc.)

The front wings are membranous, hind wings are rudimentary and are known as the halters.

The mouth parts are suitable for piercing, sucking or licking. The class *Diptera* is divided into the following chief families:—Culicidæ (mosquitoes), Muscidæ (flies), Simulidæ (buffalo gnats), Psychodidæ (moth midges), Chironomidæ (midges) and Tabanidæ (horseflies).

(A) CULICIDÆ (or the mosquitoes).—

These have three main parts of the body—the head, the thorax and the abdomen. The HEAD is sub-spherical. The EYES are large and conspicuous, forming deep notches near the insertions of the antennæ. The portion of the head behind the eyes consists of two parts, the front part being called the *frons*, and the back part, the *occiput*. The occiput is clothed with hairs. The APPENDAGES in the head are the antennæ, palpi and the proboscis. The *antennæ* are composed of 15 segments with a whorl of coarse hairs at the base, the rest of the surface being covered with numerous fine short hairs. The hairs are very numerous and much longer in the males than in the females, a very important distinguishing character between the male and the female mosquito. The *palpi* originate from the outer side of the base of the maxilla on each side and are also segmented and covered with scales. They vary greatly according to sex and tribe of the mosquito and afford a very important point in identifying them. In the Anophelini, the palpi are about the same length as the proboscis in both sexes, while in the other tribes of the mosquitoes the palpi are much smaller, often minute in the female, and very long, longer than the proboscis in the male. The *proboscis* consists of seven mouth parts, namely, the labrum (or upper lip), two mandibles, the hypopharynx, two maxillæ and the labium. During the act of biting, the labium is bent backwards near the middle, while the other organs, guided by the labella, are closely grouped together and inserted into the skin. A tube is formed by the labrum and hypopharynx through which the blood is sucked.

The THORAX consists of three segments, pro-, meso-, and metathorax, of which the mesothorax occupies the main portion, the other two segments being extremely rudimentary. The mesothorax carries the wings and legs, and the metathorax, the club-like halters, the rudiments of the second pair of wings. The *wings* are long and narrow showing numerous venations, constant for each family, and are divided into costa, running round the entire margin, the subcosta and six longitudinal veins. The *legs* are slender and long and jointed. The basal joints attaching to the thorax are the coxæ. Then come the trochanters, femora, tibiæ, and tarsi each containing five segments. The terminal tersal segment bears a pair of claws or ungues.

The ABDOMEN consists of ten segments, the last two bearing the sexual apparatus. The bodies of the mosquito are covered with *scales* showing different form and arrangement. The normal types of scales seen are the upright forked scales, curved scales and the flat scales. The EXOSKELETON consists of chitin enclosing the body cavity. The ALIMENTARY CANAL consists of three parts, namely, the foregut, midgut and the hindgut. The *foregut*, again, consists of the mouth, the buccal cavity, the pharynx, the œsophagus and the three œsophageal diverticula. The *midgut* consists of a narrow and tubular anterior portion and a dilated sac-like posterior portion called the stomach. The *hindgut* extends from the pylorus, into which open the five urinary or Malpighian tubules, followed posteriorly by the ilium and colon and finally opening into the rectum. Six ovoid papillæ project into the rectum. The SALIVARY ORGAN exists on each side of the thorax above the first pair of legs. Each consists of three tubular glands or acini, ducts and a pumping organ. The RESPIRATORY ORGANS are the chitinous air tubes or tracheæ, which ramify throughout the body. The REPRODUCTIVE ORGANS in the females consist of ovaries containing egg follicles, and oviducts with a chitinous spermatheca or seminal receptacle.

Life History.—The stages in the life history of the mosquito are the egg, larva, pupa and the imago, of which the larval and the pupal stages are aquatic in habitat.

The EGGS are small about 2 mm. in length and are deposited near or on water. The number and form vary in different tribes. The *Anopheles eggs* are separate, scattered, 50-100 in number, boat-shaped and possessing a thickened striated rim round the upper surface with structures filled up with air chambers acting as ‘ floats.’ These are deposited on the surface of water. The eggs of the mosquitoes are oval, 100-400 in number, and arranged in masses in the form of a raft. These are deposited near or on water. The egg stage of the mosquito lasts for about a fortnight.

The LARVÆ are elongated, 4-12 mm. long, footless, very active creatures. They always live in water and possess head, neck, thorax and abdomen. The HEAD carries the antennæ, two pairs of eyes and mouth parts consisting of labrum, mandibles, maxillæ with palpi and the labium. The THORAX is broad, hairy with fusion of the segments. The ABDOMEN consists of nine segments. The eighth segment bears the respiratory aperture (stigma) which opens directly on the surface in the case of the Anopheles larvæ, while it opens at the end of a long chitinous tube in the other genera. The ninth segment bears the tracheal or anal gills surrounding the anal opening. The larvæ are voracious eaters and feed on algæ, protozoa, etc. The RESTING POSITION is also characteristic in different genera. The Culex and other larvæ always hang head downwards forming nearly a right angle with the surface of water, while the Anopheles larvæ remain horizontally in the water just under the surface. The larvæ cast their skin four times before reaching the pupal stage. The larval stage lasts for one to three weeks.

The PUPA is comma-shaped, the head and thorax forming a large rounded mass with the nine-segmented abdomen curved downwards. There is a pair of breathing tubes placed on the thorax. It is very active and on account of the accumulation of air in the pupal case it remains mainly at the surface and finds it difficult to keep itself in the depth. This stage lasts for 2-3 days.

The IMAGO, or the youth adult, emerges after splitting the pupal case, which floats on the surface of water and acts as a support to the imago. The adult mosquito copulates shortly after it comes out of the pupal case and generally selects its habitat in dark protected places, *e.g.*, house corners or among foliage. They feed on various things and all are not blood suckers. The males do not suck blood, the females only being the blood-suckers. The males are short-lived (only several weeks), while the female lives for several months. *While resting*, the Anopheles remains straight owing to the head, thorax and abdomen projecting in one line. In others, the abdomen forms an angle with the head and thorax giving the hunchback appearance. The *oviposition* and *breeding* generally takes place near or on water or in places where water will accumulate at some time.

THE DISEASES TRANSMITTED BY MOSQUITO are malaria (Anopheles culicifacies, A. listoni, A. stephensi, A. fuliginosus), yellow fever [Ædes (Stegomyia) fasciata], dengue [A. (S.) fasciata, Culex fatigans], filariasis [Ædes variegata, Culex fatigans, Anopheles rossi, A. costalis].

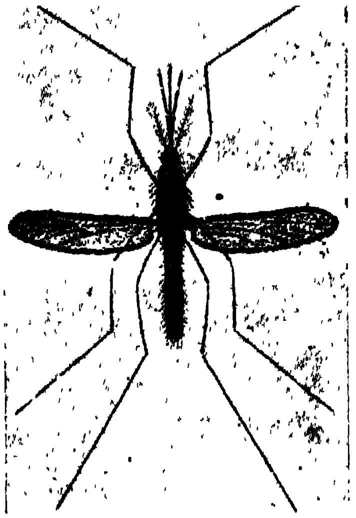


Fig. 111. Anopheles culicifacies.

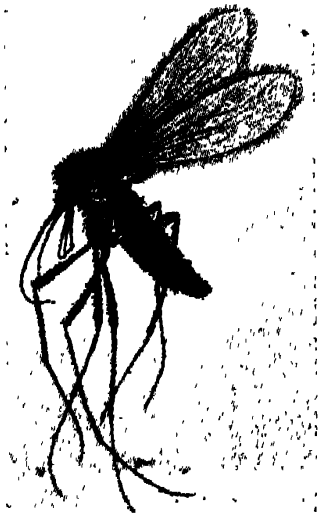


Fig. 112. Phlebotomus papatasii.

MUSCIDÆ (or flies)

These are of two kinds. Some suck blood and are provided with piercing and sucking mouth parts, while the others do not suck blood and are only provided with proboscis with no adaptation for biting. *Musca domestica* or the common house fly, as the type of non-bloodsucking diptera, possesses a single pair of membranous wings, a large pair of eyes occupying most of the surface of the head and thorax and abdomen clothed with fine hair. It passes through complete metamorphosis, *i.e.*, passes through the stages of egg, larva (generally known as maggot), pupa and the adult. The other non-blood sucking flies are the bluebottle fly, greenbottle fly, etc. (*see* also under Myiasis). The bloodsucking flies are the glossina (tse-tse fly) (*see* Figure 76), stomoxys (resembling common house fly) and the hæmatobia (horse fly).

SIMULIIDÆ (genus Simulium) or buffalo gnats.

They resemble the house fly in general appearance. They have short stout legs, large wings, short and stout proboscis, and short and straight antennæ. They are supposed to transmit pellagra.

PSYCHODIDÆ (moth midges)

They are very small in size, measuring 4 mm. in length and are very hairy. The proboscis is short while the palpi are fairly long, scaly and covered with hairs. The antennæ are long, thickly hairy and are composed of 12-16 segments. The thorax is arched and the abdomen consists of 8-10 segments The wings are large. They live in shady humid places and can fly only to a short distance, moving more or less in a series of hops. The prominent members of this family are the sandflies, Genus Phlebotomus. The common species are P. papatasii (transmits 3-day fever, dengue), P. argentipes (transmits kala-azar?), P. minutus (transmits Oriental sore), P. himalayensis etc.

CHIRONOMIDÆ (midges) and TABANIDÆ are not of much importance.

SIPHONAPTERA (fleas).

These are wingless insects, the body being compressed laterally. The head is conical with a comb of chitinous teeth along the edge of the cheek. The *mouth parts* consist of six structures, namely, the maxillæ, maxillary palpi, labium, labial palpi, mandibles and the labrum. These are suitable for both piercing and sucking. *Eyes* may or may not be present. *Antennæ* consist of three segments and are lodged in the grooves in the head, when the flea is at rest. The THORAX consists of three and the ABDOMEN of ten segments. The abdomen is the largest and the most conspicuous part of the body. The alimentary tract consists of the mouth, and pharynx which contracts and dilates rhythmically during feeding, pumping blood through the œsophagus into the stomach. The salivary ducts open into the pharynx and so the pharyngeal pump forces the salivary fluid into the wound. The alimentary canal is dilated in front of the stomach forming the proventiculus which acts as a valve and prevents regurgitation of blood from the stomach. The stomach opens into a straight tube, the small intestine, which terminates into the rectum. There are six small rectal glands. The Malpighian or urinary tubules open into the rectum Both sexes are blood suckers. The stages of metamorphosis take place in the ground. The fleas are of importance in the transmission of plague both of man and of rat. (*See* Figure 53).

RHYNCHOTA (bugs)

The HEAD carries the mouth parts which consist of a fairly long, segmented proboscis which can be flexed under the head, when not in use. These are adapted for piercing and sucking The labium forms a groove along the under surface in which lodge the mandibles and the maxillæ. The ABDOMEN is the largest part of the body. The *wings* are variable. In some there are two pairs of wings, *e.g.*, in the riduvid bugs, while in others *e.g.*, in the bed bug, there is no wing. They are blood suckers. The important families of this group of insects are (1) Cimex (Acanthia) lectularis (the common bed bug), (2) Triatoma (Conorhinus) megista, the transmitting agent of South American trypanosomiasis, and T. (C.) rubrofasciata (the riduvid bugs). (*See* Figures 71 and 72).

SIPHUNCULATA (lice)

These are small wingless insects and inhabit the external surfaces of mammals, mainly the hair. They are flattened dorsoventrally, the legs being well developed and provided with strong claws to hold the hair The mouth parts are adapted for piercing and sucking. The eggs, called nits, are laid on and remain firmly attached to the hair. The main species of human importance are, (1) Pediculus capitis (head louse—infesting the hair of the head), (2) Pediculus corporis (vestimenti), body or clothes louse—infesting the clothes and the hair of the body, and (3) the Pediculus pubis (the crab louse—infesting the hair of the public region). (*See* Figures 61 and 62.)

Apart from the irritation caused by them compelling the individual to scratch the part followed by infection and eczema-like condition of the part, these insects are known to transmit various diseases, *e.g.*, typhus fever, trench fever, relapsing fever, etc.

Crustacea (Cyclops).—Body divided into head, thorax and abdomen. Two pairs of antennæ on the head. Exoskeleton hard due to impregnation of calcium salts.

Myriapoda (Centipedes and millipedes).—Body divded into a head and a large number of segments.

A. Venomous arthropoda.—Bees, wasps, beetles, scorpions, etc.

They possess a highly poisonous salivary secretion, the poisonous constituent being protein in nature. It is a clear, faintly acid, and more or less oily fluid used for the purpose of defence as well as to kill the prey. The toxicity is sometimes so high as to cause death in small birds within a very short time varying from 2 to 30 minutes.

B. Diseases caused by arthropoda.

Myiasis.—This is caused by the destructive action on tissues by the larvæ of some dipterous insects This is of three forms:—

(*a*) MYIASIS OF LESIONS:—External myiasis.

This is of commoner occurrence. The flesh flies (sacrophagidæ), the house fly (muscidæ), screw-worm fly (chrysomyia) and allied insects ocasionally feed and lay eggs on the exposed or neglected wounds of debilitated persons, or during sleep. The eggs develop into maggots which cause severe destruction of tissues with often fatal results.

(*b*) INTESTINAL MYIASIS. This is less common than the preceding. The eggs of the above insects are swallowed by man and develop into larvæ, large numbers of which may be discharged through the fæces. Their presence in the alimentary tract may occasion severe irritation of the intestines.

(*c*) MYIASIS OF THE TISSUES. The larva of some flies, *e.g.*, Dermatobia hominis, the so-called *Tumbu-fly*, burrows under the skin leaving an opening in the skin, through which it breathes.

C. Diseases transmitted by the arthropods.

1. Malaria (Anopheles). 2. Filariasis (Culex, stegomyia). 3. Dengue (Culex and stegomyia). 4. Yellow fever (Stegomyia). 5. Guinea-worm (Cyclops). 6. Calabar swelling (Biting fly). 7. Plague (Rat fleas). 8. Relapsing fever (Tic and louse). 9 Trench fever (Louse). 10. Typhus fever (Louse). 11. Sandfly fever (Sandfly). 12. Trypanosome (Tse-tse fly). 13. Chagas disease (Conorhinus). 14. Japanese river fever (Mite). Kala-azar(?)

THE MORE IMPORTANT INSECT PARASITES OF MAN

Phylum.	Class.	Order.	Family.	Genus.	Species.	Diseases caused or transmitted by them.
Arthropoda	Arachnoidea	Acarina	Sarcoptidæ	Sarcoptes	S. scabiei **(mite)**	Scabies.
			Demodicidæ	Demodex	D. folliculorum;	Itch.
			Ixodidæ	Ornithodorus	O. savignyi **(tic)**	Relapsing fever and typhus.
				Dermacentor	D. andersoni	
	Insecta	Diptera	Culicidæ	Culex	C fatigans **(mosquito)**	Filariasis, dengue.
				Anopheles	A. maculipennis	Malaria.
				Aedes	A. fasciata	Filariasis, dengue.
			Psychodidæ	Phlebotomus	P papatassi **(sandfly)**	Sand-fly fever.
			Simulidæ	Simulium	S. reptans	Pellagra.
			Muscidæ	Glossina	G. palpalis **(tse-tse fly)**	Sleeping sickness.
					G. morsitans	Do.
				Musca	M. domestica **(fly)**	Cholera, dysentery, etc.
				Tabanidæ	T. bovinus	
		Sihonaptera.	Pulicidæ	Pulex	P. irritans **(flea)**	Plague.
				Xenopsylla	X. cheopis;	Plague.
		Rhynchota	Acanthiidæ	Acanthia	A. lectularia **(bed bug)**	
				Conorhinus	C. sanguisugas **(riduvid bug)**	Chagas disease.
		Siphunculata.	Pediculidæ	Pediculus	P. capitis **(lice)**	Relapsing fever, typhus fever and trench fever.
					P. pubis	
					P. vestimenti	
	Crustacea			Cyclops		Guinea-worm.
	Myriapoda				**Centipedes & Millipedes.**	

D. Diseases sometimes transmitted by insect.

These are cholera, dysentery and typhoid carried by all flies that haunt food and fæces. Pyogenic infection, anthrax, leprosy, favus are transmitted mechanically by contact from the sick of the healthy.

THE METHOD OF TRANSMISSION OF THE COMMUNICABLE DISEASES

1. Direct. 2. Indirect. 3. Through some intermediate host. and 4. Unknown.

1. Direct method. This generally takes place when the skin or mucous membrane of healthy persons comes in direct contact with the affected part of the patient, as in cases of syphilis, gonorrhœa, scabies, small pox and other contagious diseases. The virus of rabies is directly introduced into the tissues of man or other animals by the infected one.

2. Indirect method. This takes place when there is some medium interposed between the source of infection and the part of the individual to be affected. This medium generally favours the growth of the infecting organisms, or at least sustains their vitality for a fairly long perod. The media acting as common modes of conveyance are the air, water, soil, food, fomites etc

Air acts as the transmitting agent in cases of infcetion through the pulmonary passages, such as tuberculosis, pneumonia, influenza, diphtheria, pneumonic plague, etc. In these conditions the infective organisms leave the patient through minute particles of sputum during coughing and float in air, until they settle on the mucous membrane of the respiratory passages, or on the dust of the floor, which again will act in the same way when disturbed.

Water acts as the transmitting agent in the so-called water-borne diseases, such as cholera, typhoid, dysentery, etc. The organisms gain access to the water from contamination thereof with the evacuations of the patient. They may remain alive in the water even for months unless the latter is properly disinfected.

Soil transmits diseases, *e.g.*, tetanus, gas gangrene, etc.

Food such as milk or other drinks, meat, vegetables taken raw may form the vehicle for carrying diseases. Milk is usually contaminated during collection or distribution by the addition of polluted water used to adulterate it or to wash the containers, or infected if drawn from diseased animals. Milk often acts as the transmitting agent for diseases, *e.g.*, tuberculosis, malta fever, etc. Meat, when diseased and imperfectly cooked so that the organisms are not destroyed, transmit diseases, *e.g.*, tuberculosis, botulism, worms, etc.

Fomites.—The use of infected clothings, beds, cups and other utensils leads to the transmission of the infection to the healthy with production of disease.

3. Through intermediate host. This may be, (*a*) Mechanical. (*b*) Indirect. (*c*) Direct.

(*a*) *Mechanical.*—In this case, the agent acts as the mechanical carrier by conveying the disease germs on their persons from the source to food or elsewhere. Here, the organisms *do not pass through any cycle of development in the body* of the agent. This is commonly illustrated in cases of mechanical transmission of typhoid, cholera, dysentery, etc., by means of the flies.

(*b*) *Indirect.*--Here, the organisms are taken into the gut or other parts of the body of the insect, where they remain in an infective condition for a varying length of time, occasionally multiplying enormously, *without passing through any cycle of development.* The transmission of plague, trypanosoma, relapsing fevers, typhus, etc., may be cited as instances.

Human beings often act as carriers during (*i*) The period when they are suffering themselves. (*ii*) Convalescence. (*iii*) By contact with a patient but not suffering themselves.

(*c*) *Direct.*—In this instance, the organisms *pass through a cycle of development* in the host as in cases of malaria, filaria, etc. It is a noticeable fact, that some species of insects are always selected by the organisms as the host for their transmission. Malaria parasites will always be transmitted by certain species of anopheles, and by no other. The same is also true of other diseases transmitted similarly.

4. Unknown.—As in kala-azar (for discussion see page 206),

CHAPTER XIX

SNAKES

Snakes belong to the class REPTILIA and the Order OPHIDIA and are generally divided into Families Colubridæ (Colubrine snakes) and the Viperidæ (Viperine sankes). The HEAD may be elongated and narrow (Colubridæ) or triangular and stumpy, (Viperidæ). The TEETH bear the most important characters for distinguishing between poisonous and non-poisonous snakes. In some small worm-like snakes, there is only one jaw carrying teeth while in all the other families both jaws carry teeth. In the non-poisonous snakes, the teeth are solid, small, sharp, more or less directed backwards and the outer or the maxillary teeth extend along the whole of the gum margin. In the poisonous snakes, the upper marginal row of teeth does not exist. Its place is taken by the poison fang which is large, grooved or canaliculated, and occasionally curved. The remainder of the gum margin remains bare of teeth. The poison from the poison gland is injected into the wound made by the fang through the groove or canal inside the fang. The SKIN is covered with scales which form the main characteristics for the identification of the snakes and are generally grouped under three heads,--head scales, body scales and tail scales. The size and distribution of the head scales are shown in the accompanying figures. The COLOUR of the snakes depend mainly on the colour of the scales. The colour of the snakes is very variable and is often a fallacious guide for identification. The RIBS of the neck are long in some groups and can be stretched out at right angles so as to form the well known hood. The TAIL of the sea snakes is compressed like a rudder.

Snakes are divided into nine families:—

A. Small worm-like snakes generally living underground—1. Typhlopidæ, 2. Glauconiidæ.

B. Non-poisonous snakes—3. Boidæ (pythons, boas etc.), 4. Ilysiidæ and 5. Uropeltidæ the last two being burrowing earth snakes. 6. Amblycephalidæ, 7. Xenopetidæ and 8. Colubridæ (series Aglypha and Opisthoglypha).

C. Highly poisonous snakes—8. Colubridæ (series Proteroglypha) and 9 Viperidæ. Russell's viper and rattlesnakes belong to this last group.

The most important snakes in India are the cobra (Naja tripudians), the king cobra (Naja bungarus) and the Kraits (Bungarus fasciatus).

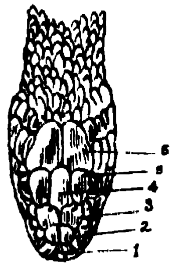


Fig. 113.

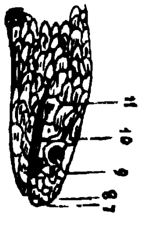


Fig. 114.

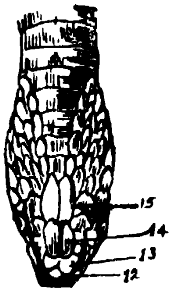


Fig. 115.

Fig. 116.

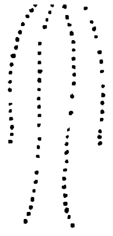


Fig. 117.

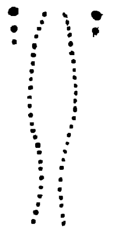


Fig. 118.

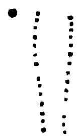


Fig. 119.

Fig. 113. Colubrine head viewed from above. Fig. 114 The same viewed from side. Fig 115. The same viewed from below Fig. 116. Viperine head.

Head scales---1 rostral, 2 and 3 frontals, 4 parietals, 5 supra-oculars, 6. occipitals, 7 prenasal, 8. nasal, 9 post-nasal, 10 post-oculars, 11. supra-labials, 12. mental, 13 infra-labials, 14 submentals, 15 linguals

Fig. 117. Diagram showing the mark of the bite of a non-poisonous colubrine snake Fig. 118 The same of a poisonous colubrine snake Fig. 119. The same of a poisonous viperine snake The thick dots indicate the mark of the poison fangs.

The Nature of the Toxic Substances in the Venom.—

The toxic substances vary in different snakes and are mainly (1) the *neurotoxin,* which is thermostable and resides mainly in the albumoses, (2) the *hæmorrhagin,* which is thermolabile and resides in the coagulable proteins, (3) the *anticoagulin,* which prevents coagulation of blood both *in vitro* and *in vivo* and (4) *thrombase,* which produces rapid coagulation of the blood either locally or throughout the body.

The Action of the Snake Venom.—

This depends on the variety of the snake, whether colubrine or viperine; the size of the snake and the quantity of the venom ejected. One adult cobra generally yields about 1 ccm. of venom or about 317 mgm., the minimum lethal dose for man being about 15 mgm. It is note-worthy that the snakes, even the deadly ones, do not always eject venom into the wound. Thus cases are on record in which no poisoning effect was noticed after the bite of highly poisonous snakes. The amount of poison also diminishes after each bite. It has been recorded that after a cobra bite to four persons successively,

The bite of the colubrine snakes is less painful and is soon followed by local loss of sensibility. The absorption of the venom, the toxic principle of which is mainly neurotoxin in nature, into the general circulation leads to general loss of consciousness with difficulty of respiration and slow pulse. Death takes place in a comatose condition with paralysis of muscles especially those of respiration, tongue and face. Cobra venom is highly hæmolytic. Blood remains fluid and coagulation is greatly delayed.

The bite of the viperine snake, on the other hand, is painful. The part becomes red and then violet with hæmorrhages in the surrounding tissue followed by gangrene. Dryness of the throat and mouth takes place and is accompanied with great thirst. Systemic absorption of the venom leads to hæmorrhages in different organs notably the eyes, alimentary tract, and the urogenital tract. Death takes place from vasomotor and respiratory failure. Blood is very rapidly coagulated and intravascular thrombosis may take place. The toxic principles are mainly hæmorrhagin and thrombase.

CHAPTER XX

INCIDENCE OF INFECTIOUS DISEASES

With Special Reference to India

The following are the most common important infectious diseases prevalent in India:—Malaria, kala-azar, cholera, small-pox, tuberculosis, hookworm infection, filariasis, leprosy, beriberi, plague, dengue, dysentery, etc.

MALARIA

It is the most common disease prevalent throughout India.

History.—It was introduced in the fifth century B.C. in the Roman army while passing through the Pontine marshes. Reference about connection between malaria and mosquitoes can be found in *Susruta.* Cinchona bark as antidote to malaria was discovered in 1640. Pigments in the blood were discovered in 1847. Laveran noticed amœboid movements in the corpuscles in 1878. Danelesky found plasmodium in birds in 1885. Ross worked with culex (1894-96), then tried anopheles in 1896 and found zygote in the stomach. Other workers, *e.g.*, Grassi, Koch and others, completed the cycle independently.

Distribution.—A. *Hyperendemic.*—(*i*) In the hill tracts.—The Terrai and the Dooars, the Western Ghats, the Eastern Ghats (probable), parts of C. P. and Orissa, Assam, Chittagong hill tracts, Jaipur hills, Madras. (*ii*) In the plains.—Bengal—Nadia, Jessore, parts of 24-Parganas, Burdwan, central Bengal.

B. *Epidemic malaria.*—In the Punjab, Rajputana, Sikarpur, a small part of the north-west of C. P., northern part of U. P.

C. *Endemic malaria.*—Specially in U. P., lower part of the Ganges valley, Behar, C. P., Madras, North-West Frontier, Rajputana, parts of the Punjab.

Definition.—*Spleen index*—percentage of palpable spleen—usually examined in children between 2 to 10 years of age—preferably taken in standing posture.

Hyperendemic—spleen index—Above 50 per cent.
Moderately endemic—spleen index—15-50 per cent.
Low endemicity—spleen index 10-15 per cent.
No malaria—spleen index—below 10 per cent.

There is no epidemic malaria if there is no endemic malaria (either low or high). There is no malaria at over 5,000 ft. in this country.

Malaria Mortality.—In Bengal 11.3 per 1,000. In India every year 1½ million deaths take place from malaria In Bengal, alone in 1925, half a million deaths took place.

The number of persons infected is roughly about 30,000,000 (out of 74,000,000 in the whole Bengal).

The follownig *factors* are necessary to carry infection of malaria:—

1. People must be infected—the human factor. 2. Anopheline factor. 3. Climatic conditions.

Some important fundamental factors regarding the malarial parasite and its transmission:—

1. Varieties of the parasites, B.T., Q.T., M.T., development of gametocytes after 8-10 days of fever. 2. Incubation period—average 14 days. 3. Mosquito development—egg to larva—15 days; larval stage 8-10 days; pupa stage 1-2 days; then adult mosquito. 4. Sporozoite formation in the mosquitoes—10 days after feed. So sequence of events are:—Rains during June, July, August—followed by breeding of mosquitoes—followed by infected mosquitoes—infected persons—death.

So antimalarial measures must be taken at least 1½ months before the malaria season begins, which lasts from September to December (maximum).

A. *Human factor in the transmission.*—(*i*) Some population infected with *parasite;* (*ii*) Some population not infected with *parasite and gametocyte;* (*iii*) Some population not infected but susceptible. All these factors play a very important part in the transmission.

B. *Anopheline factor.*—Types of mosquitoes most prevalent in Bengal which act as natural carriers are:—A. maculatus, A. listoni, A. culicifacies, A. stephensi in Calcutta. Doubtful carriers:—A. fuligenosus, A. rossi.

Malaria always tries to go up to a certain level and stay there (Ross). When you reduce your anophelines to a certain level—*e.g.*, 10 per head—malaria curve goes down. Malaria also disappears gradually, after attaining a constant low level. It is not necessary to kill any and every mosquito, but it is necessary that the balance must be kept down.

If the number of mosquitoes per individual be less than 10, malaria slowly disappears. About these mosquitoes—biting chance is 1 in 4, *i.e.*, 1 out of every 4 mosquitoes may bite. Of those which bite some only survive after the bite and form sporozoites in the salivary glands, chance 1 in 3. Only some of these, again, bite after the formation of sporozoites, chance 1 in 4.

Conditions necessary for mosquito development.—Clear shallow water with certain amount of vegetation near the banks. Rainy season is the breeding season. If the mean temperature is below 60°F—very little mosquito development takes place.

Conditions necessary for sporozoites development in the mosquitoes.—Suitable temperature about 18°C. and humidity.

Relations between Malaria and Rainfall.

In the Punjab, malaria curve rises with increase of rainfall. In the case of Bengal, though in the beginning the curve rises with the increase of rainfall, it falls down if the increase of water continues.

In Bengal, the places which are comparatively dry and elevated suffer most from malaria—because it is a deltaic plain, rainfall is excessive and one cannot dry up the entire land during the rainy season; so when rain falls on a comparatively dry elevated region, it forms pools with large amount of water-edge which are the breeding places of mosquitoes. So in these circumstances attempts at reducing the amount of water on the land are almost certain to be followed by an increased production of anopheles and increased malaria.

Immunity.—There is no acquired immunity but tolerance. This tolerance is particularly to be found among the aborigines of Bengal, *i.e.*, Santals, female coolies in the tea gardens, etc. Age distribution has certain effect on this immunity as shown by the spleen index. Between 2—4 years, spleen index may be 100. Between 4—10 years, spleen index is high but malaria less. After 10 years, marked immunity.

Anti-Malarial Measures.—*Human factors.*—(*i*) Reduce the number of infected person—early diagnosis and early treatment. Protect the susceptible persons. (*ii*) Avoidance of malarious sites and places. (*iii*) Personal prophylaxis—mosquito-nets, quinine prophylaxis, mosquito repellants, etc.

Anopheline factors.—(*i*) Reduction of surfase water *e.g.*, in hill tracts. (*ii*) Increase of surface water (temporary floodings)—most suitable in Bengal. (*iii*) Drainage system—subsoil drain, surface drain, contour drain—not suitable for Bengal. (*iv*) Local works—jungles at the edge of the pools to be removed, edges to be cleaned, filling up of hollows, stream training (canalisation), kerosinising and use of Larvicides—kerosine oil 30 gallons for 1 acre by spray or drip can, etc.

KALA-AZAR

History of the Disease.—In 1835, Twining observed Cancrum oris. In 1854, Burdwan fever spread from Jessore to the whole of Burdwan. In 1872, it spread along Rangpur, Dinajpur to Assam Valley. In 1913, Sibasagar was reached. After this it became endemic in the whole of Assam.

In 1882, a definite clinical description of the disease was given by Clarke. In 1903, Leishman discovered the parasite in the spleen of a soldier at Dumdum. Donovan also found it in the same year in Madras. Hence it is called the Leishman-Donovan body.

Antimony treatment was started in Italy in 1915. Kala-azar has probably been brought from Africa by traders to Bengal.

Distribution.—India, Yang-set-kiang Valley in China, Sudan, coasts of the Mediterranean Sea.

Other forms of Leishmaniasis.—Tropical sore in the Punjab, Egypt, Palestine, America; in Bengal—24-Parganas, Jessore, Rangpur, Mymensing are highly endemic; Burdwan, Bankura less.

Incidence.—In 1920, Sur found in every village an average number of infected persons to be 2; there are 25,000 villages in Bengal, so 50,000 persons are affected. Stewart believes in the presence of 200,000-500,000 cases in Bengal.

Transmission.—*Prevention.*—Difficulties as the vector is not known. Sandfly is suspected. However following measures may be adopted:—(1) K. A. dispensary—free distribution of medicines and free injections. (2) Isolation of the cases. (3) Disinfection of the excreta of the cases. (4) Clearing away of jungles, filling up of low-lying lands. (5) Personal prophylaxis.

CHOLERA

History.—*Pandemics.*—First it started in Bengal in 1817. In 1826, it spread from India to Mesopotamia.

Big outbreaks.—1826 in Bengal, 1827 in the Punjab, 1828 in Afghanistan, 1829 in Persia, and reached Russia in 1831.

Another outbreak.—Hardwar in 1866, reached Afghanistan in 1867, Persia in 1868, and Europe in 1870.

Incidence.—No part of India is free from Cholera. It occurs endemically in India in Bengal, Behar, Assam, S. E. Madras and Bombay coast. It occurs epidemically in Bengal (24-Parganas, Mymensingh, Jalpaiguri and many other places, specially after fairs and melas), the Punjab, the United Provinces and the Central Provinces.

Infection in an epidemic area comes from endemic foci.

Mortality	Ordinary years	Epidemic years.
India	0.3 per 1,000	1.2
Bengal	0.9 „ „	3 to 5
Behar	0.26 „ „	2.2
U. P.	0.06 „ „	1.48
The Punjab	0.01 „ „	2

Every year 40,000 deaths occur in Bengal. In 1924, 2,76,567 deaths took place in India with 41,899 in Bengal Cholera is declining in Bengal.

Seasonal Prevalence.—*Bengal*—Starts in January, drops in February, goes up from latter part of February, maximum in April and then declines. There is another rise in December. *Behar* (Patna)—Two peaks in July and August merged into one peak. *Madras*—There are two peaks, one in July and the other in December.

Types of Bacilli.—At the times of the epidemics, one finds agglutinating strains at the height of outbreak and non-agglutinating strains at the decline of the epidemic.

Modes of Transmission.—This is spread by man from place to place through direct and indirect contact. It follows the lines of trade and travel. Outbreaks take place mainly from pilgrims contaminating water and food supply. Carrier and fly also take great part in the transmission of the disease.

Immunity.—This lasts only for a short time (about 14 days). Immunity after Haffkin's inoculation lasts from 6 to 14 months.

Preventive Measures.—*Personal prophylaxis.*—Boil water and milk, cook vegetables, avoid bazar-made foods, take inoculation and treat dyspepsia if present.

Domestic measures when a person is ill.—

(1) Notify to the health authorities; (2) isolate the patient; (3) disinfect fæces with bleaching powder (5 per cent.), quicklime, izal (1 in 200), cyllin (1 in 150), etc.; (4) disinfect utensils used by the patient; (5) boil water and milk; (6) inoculate other members; (7) disinfect the whole house specially the latrines; (8) protect the house and food from flies

SMALL-POX

History.—Known from ancient times. Reference can be found in *Charaka* and *Susruta Samhita.* In China this is known as "Bean disease." It entered Europe from the East the exact date of which is unknown, and spread to France and Italy in 6th Century A.D., to Great Britain in the 8th century and to America in the 16th century.

Incidence.—India—76,000 die annually. Bengal—15,000 die annually. In epidemic times the mortality 0.4, and during non-epidemic times 0.1, per 1,000.

Seasonal incidence.—March, April and May are the months of greatest small-pox intensity, heaviest mortality in April. Least prevalence from September to November. In Bengal small-pox is epidemic every 5th year, and every fourth recrudescence appears to exceed the intermediate ones in intensity and severity. In Bengal, small-pox districts are divided into 2 main groups—in the West group Calcutta is the centre, while in the N. E. group Kamrup is the centre.

History of the Development of Vaccination.—It was discovered by Jenner in England. The first inspiration came from a young dairy maid in 1768: "As I had cow-pox, I cannot take small-pox." The first crucial experiment took place in 1796, while in 1802 the final crucial experiment was done.

Immunity from Vaccination.—This appears about the 8th day of vaccination. Definite protection lasts, on an average, from 5 to 7 years after successful vaccination.

Modes of Spread.—It is highly infectious and is disseminated by infected persons through direct and indirect contact. Air-borne theory is also advanced. The virus which is present in the skin lesions and secretions from the mouth and nose, are harboured and transported by fomites, *i.e.*, by towels, handkerchiefs, spoons, etc. It is most infectious during the vesicular, pustular and scabbing stages and is contagious even in the pre-eruptive stage.

Incubation Period.—Is 12 days on the average.

Preventive Measures.—(1) Inform the health authorities; (2) vaccination of the healthy; (3) isolation of the sick; (4) disinfection of excreta and utensils; (5) fixation of contagious matter by smearing antiseptic oil on the skin; (6) quarantine for 14 days.

TUBERCULOSIS

Incidence.—In Bengal it is on the increase, yearly death over 4,000. Most noticeable amongst the young females and young men particularly those inhabiting the urban area. There are about 150,000 persons suffering from tuberculosis.

Modes of Spread.—Spread from man to man through direct association and from cattle to man.

Prevention.—Prevention of tuberculosis has now become a sociological problem. Poverty with its attendant hardship, poor food, bad housing, overcrowding, overwork, mental worry, diminished resistance, are mainly responsible.

Preventive Measures.—There are 2 categories, (*i*) avoiding the infection by segregation, use of tuberculosis tested milk (not common) or pasteurised milk, education, disinfection, proper disposal of sputum, avoidance of contact with open cases, and early diagnosis.

(*ii*) Increasing the resistance of the individual through personal hygiene by means of fresh air, good food, rest, personal hygiene; sociological and economic reforms, *e.g.*, improvement of housing condition, lowering the cost of living, all forms of uplift and help, abolition of early marriage and purdah; establishment of certain secondary agencies, *e.g.*, preventive clinics, industrial notification, open air school, etc. The number of persons suffering is ten times the number of deaths. The number of beds necessary is twice the number of deaths. In Bengal there are only 92 beds for tuberculosis cases.

A Scheme for Prevention of Tuberculosis is given below:—

The duties lie on the *Doctor, Health authorities, the State and the Public*:—

Doctor ..
- Home treatment.
- Sanatorium treatment (after cure return to work).
- Notify to the Health authorities.

Health authorities
- Tuberculosis register.
- Tuberculosis dispensary
- Health visiting committee.
- After-care committee.
- Isolation hospital (1 for 5,000 persons).
- tuberculosis colony.
- diagnosis of cases.
- educative efforts.
- examination of contacts.
- tracing of the infection.

The State and the Public.
1. Environmental improvements (housing, good food, etc.).
2. Educational efforts.
3. Improvement of industrial hygiene.
4. Open-air schools for tuberculosis children.
5. Improvement and control of milk.
6. Social reforms.

LEPROSY

Incidence.—In India– Bengal, Bombay, U P. and Madras.

Asylums are situated in Bengal at Gobra, Raniganj (missionary), Bankura (missionary), (proposal at Midnapur); Bombay at Mutra; U. P. at Almora; Madras at Chingleput. Case incidence in Bengal is about 17,000.

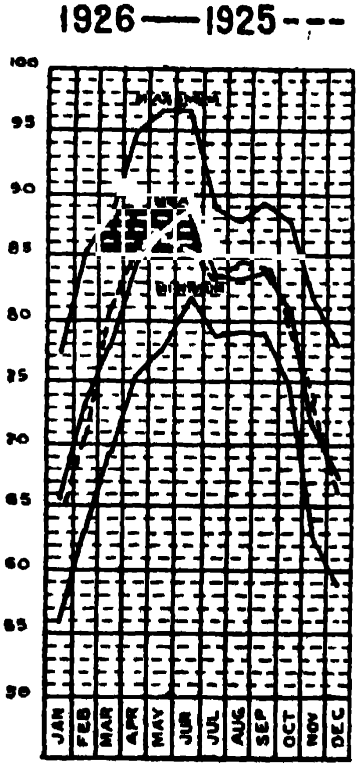


Fig. 120. Calcutta-Temperature.

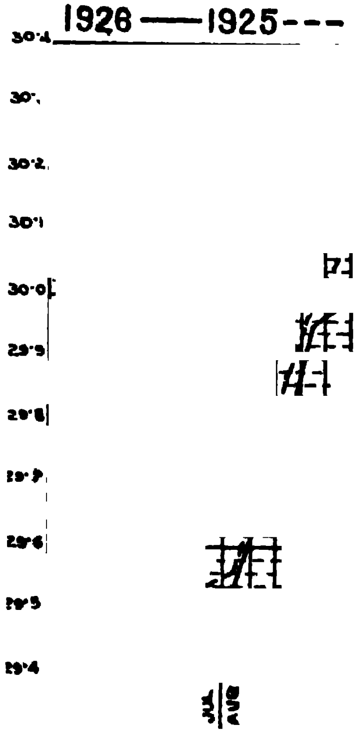


Fig. 121. Calcutta-Barometer reading.

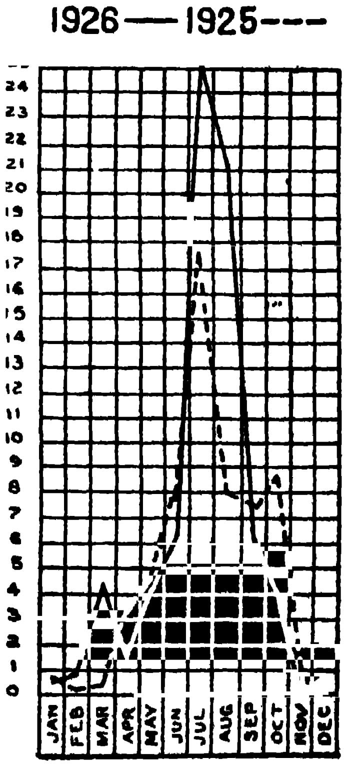


Fig. 122. Calcutta—Rainfall in inches.

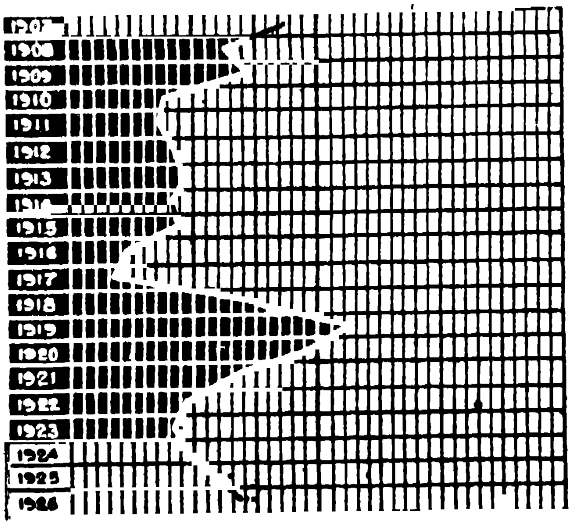


Fig. 123. Total mortality for 20 years.—Rate per 1,000 population. (Calcutta)

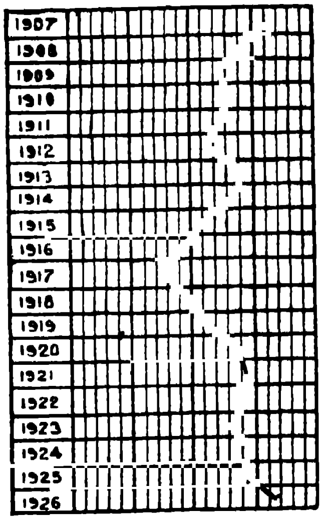


Fig. 124. Tubercle mortality for 20 years.—Rate per 10,000 population. (Calcutta)

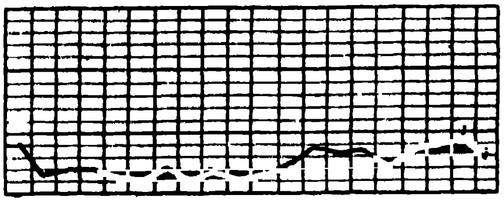


Fig. 125. Fever.

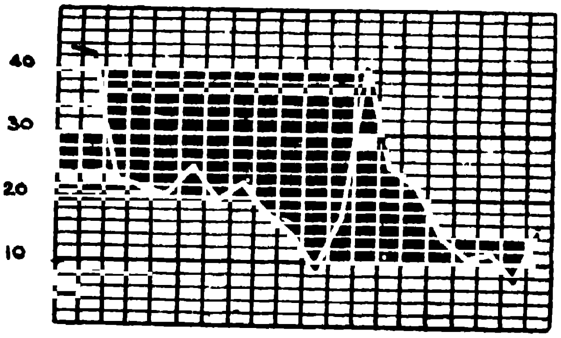


Fig. 126. Small-pox.

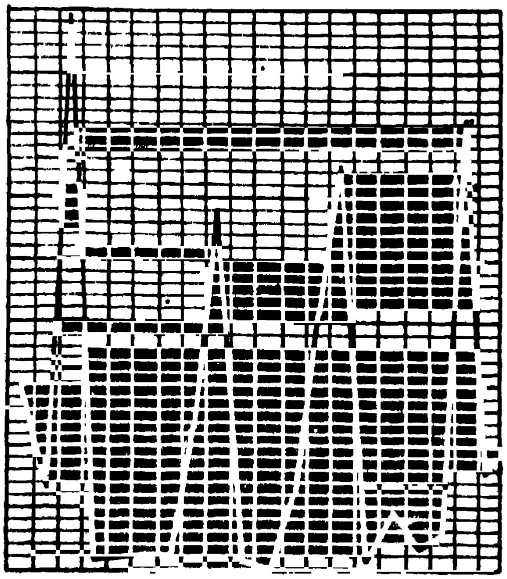


Fig. 127. Cholera.

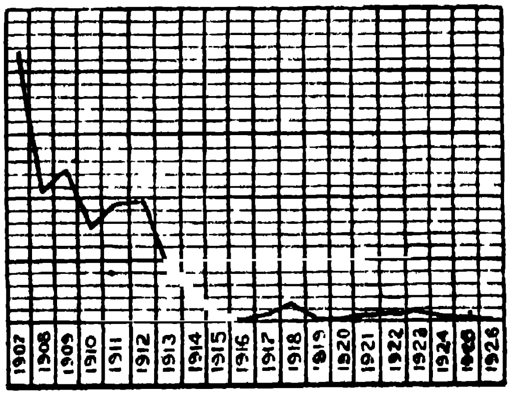


Fig. 128. Plague.

Preventive Measures.—A system of (*i*) Avoidance of contact with lepers. (*ii*) Voluntary notification—to early diagnosis and efficient treatment. (*iii*) Voluntary segregation in voluntary colonies. (*iv*) State regulation prohibiting a leper in certain occupation.

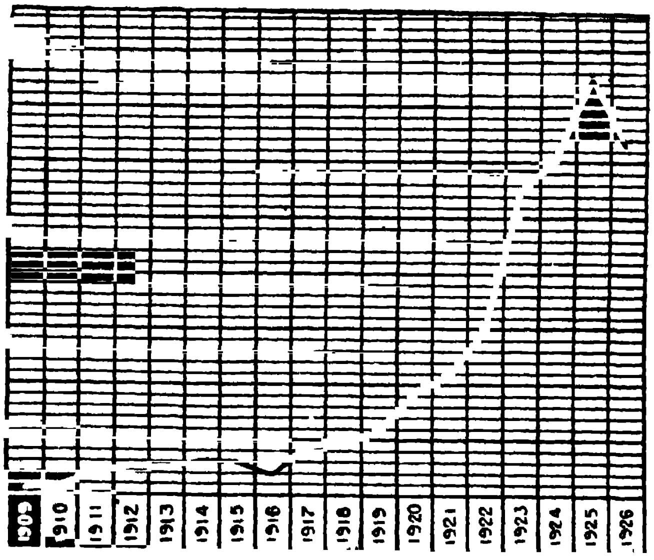


Fig. 129. Kala-azar.—Rate per 1,000.

Figures showing mortality from infectious diseases in Calcutta for twenty years. (Rate per 10,000 population (except Kala-azar).

(Figures 120 to 129 taken from the report of the Health Officer of Calcutta for the year 1926—by kind permission.)

PLAGUE

History.—First record in Europe is found in the latter half of 6th century. Epidemic in 14th century in Europe known as the **Black Death.** In 16th century, a terrible pestilence depopulated China. In 17th century, severe outbreak occured in Italy. In 18th century, Eastern Europe was affected. In 19th century, Constantinople, Egypt, Crimea and Caucasus were affected. The first outbreak recorded in India was in 1612 during the time of Emperor Jahangir. In 1815, Gujerat, Kathiawar and Cutch were affected. In 1836, Rajputana was affected. In 1894, an outbreak took place in Canton, thence to Hongkong. Bombay was affected in 1896 either from Hongkong or from Benichair in Arabia or from Kumaon on the southern slope of the Himalayas.

Seasonal incidence.—Places which are endemic have seasonal prevalence, but intensity varies. Lowest incidence in July, maximum intensity in February, March, April. Low temperature and high humidity (particularly when winter is prolonged) have important effect on the incidence. Onset of real hot weather lessens the incidence.

Bengal—only a few sporadic cases. *Behar*—starts in February, maximum in March. *U. P.*—maximum in April. *C. P.*—maximum in March. *Bombay*—maximum in November. *The Punjab*—commences in November and December. *Burma*—varies. Endemic areas are Behar, U. P., Punjab, C. P., Burma, Madras (very slight), Bengal (no endemicity).

Factors in the Spread.—Rats and rat fleas.

Types of rat.—Mus. rattus (house rat), commonest carrier. Mus. decuminus (sewer rat), comparatively rare in India. First plague occurs amongst the sewer rats which keep up the infection, followed 15 days after by plague among mus. rattus. This is followed, 7 to 15 days after, by plague among men.

Rat fleas are of 2 types.—(*i*) Xenopsylla Astia—which keep on infection. Common carrier according to Col. Taylor. (*ii*) Xenopsylla cheopis—which spread the infection.—*Dr. Hirch's view.* Before 1898, X. Astia was the main flea in India. After 1896, X. Cheopis imported from Egypt, the home of bubonic plague. Fleas can jump to a height of 4 inches and can hop 30 yds. on legs. Plague spreads from rat to man through agency of rat flea.

There are two theories:—(1) Fleas continually discharge the contents of their intestine during the act of sucking. The infected fæces rubbed in by scratching in the neighbourhood of puncture affords possible means of infection. (2) The bacilli multiply in the stomach of the flea. During the act of sucking, the contents are regurgitated in the wound.

Preventive Measures.—(1) Compulsory notification and registration of all cases of death. (2) Isolation of the sick. (3) Thorough disinfection of the clothing of the infected person and also the infected household. (4) Houses in which dead rats have been found are to be disinfected. (5) Protective inoculation—Immunity for 1 month. Attack rate reduced between 52 to 94 per cent. Death rate reduced between 75 to 100 per cent. (6) Improvements of insanitary areas. (7) Complete system of scavenging. (8) Free Medical Relief. (9) Rat proof dwellings, godowns and systematic deratisation. (10) Education of Public. (11) Ry. Inspection and Segregation Camps. (12) Forbidding exportation of rags from an infected district. (13) Quarantine. (14) Passport. (15) Evacuation

HOOKWORM (Discovered by Loose in Egypt)

Distribution.—Hot and moist places are affected. Tropical and Subtropical, diminishes towards temperate climates. The following countries—India, America, Australia, found near coal mines in different parts of Europe. Bengal—excepting Assam (heaviest), Terrai and Jalpaiguri—is not heavily infected.

Species.—Ankylostoma duodenalis—Coal miner's itch. Necator Americanus—More common in Bengal.

Intensity of infection is gauged by the number of eggs per gram of fæces. Slight infection means 40 eggs to 7,500 eggs per gram or 1 to 200 worms. Moderate infection—7,500 to 15,000 eggs per gram of fæces. Heavy infection—Over 15,000.

Immunity.—A definite racial immunity in Negroes and Filipinos. No acquired immunity. Hookworm disease lowers the vitality and predisposes to other infections—comonly Tuberculosis.

Prevention.—Prevention depends upon the social and economic uplift of the nation, mass education, change in habits of the people. These are (1) provision of proper privies and insistence on their use; (2) education by lantern lectures, pamphlets, exhibits; (3) duties of the Medical Profession; (4) personal prophylaxis—use of shoes, gloves, etc.

CHAPTER XXI

URINE ANALYSIS

Collection

For *qualitative* examination the morning sample is sufficient. For *quantitative* examination, the specimen to be examined should be a portion of the mixed urine passed in 24 hours. Preserve the urine from decomposition by adding a few drops of formalin, or a few crystals of thymol, with the first sample. Measure the total quantity of urine during 24 hours.

Normal constituents

Water 1,200; *Total substances in solution* 60; *Inorganic substances* 25; Chlorides (chiefly NaCl) 12.5; Phosphates (earthy 1 alkaline 2), total 3; Carbonates, nitrates, silicates, etc. traces; Ammonia 0.7; *Organic substances* 35; Urea 30; Uric acid 0.7; Creatinin, hippuric acid, purin bases, oxalic acid, fatty acids (volatile), pigments, acetone, etc. traces.

Urea is the most important constituent of urine, from the physiological point of view. This constitutes the principal waste-product of metabolism and forms about one-half of all the solids excreted, and represents about 90% of the total nitrogen of the urine. *Pathologically,* urea is increased in fevers, diabetes, during absorption of large exudates and resolution of pneumonia. A decrease occurs either due to decreased formation or diminished excretion. Its formation is decreased in diseases of liver with great destruction of the hepatic tissue, *e.g.,* in cirrhosis, cancer, acute yellow atrophy, etc. Diminished excretion occurs in nephritis.

Physical Examination

I. Quantity.

This can be decided only when the total amount passed in 24 hours is available. A healthy man passes normally about 50 fluid ounces of urine in 24 hours. *In health,* the daily amount is increased (*a*) after copious drink, (*b*) in cold weather by diminution of sweat. Decrease occurs from the opposite conditions. *In disease,* it is increased, (1) in diabetes mellitus and insipidus, (2) in arteriosclerotic kidney, (3) with high blood pressure, (4) in hysteria, and (5) during the excretion of exudates, *e.g.,* dropsical effusions. Decrease occurs, (1) in acute and chronic nephritis. (2) in diarrhœa. (3) in fevers. (4) with low blood pressure. (5) after loss of blood (*e.g.,* hæmorrhage) or of fluids of the body (*e.g.,* cholera).

II. Colour.

Normal urine is of pale yellow colour. The colour is pale when the amount of urine is excessive as in diabetes. Urine is darker when the amount is scanty.

A reddish or orange-brown tint may indicate blood or the use of drugs such as rhubarb, senna. The urine, with addition of alkali, turns bright pink or red due to presence of phenolphthalein taken as laxative. Greenish or greenish-black urine may be due to bile, santonine, or, to the use of carbolic acid or other coal tar derivatives. A yellowish tint may be caused by bile, and milky appearance by fat, chyle or pus. The urine may appear blue in typhus fever, or from methylene blue; opalescence may be due to the presence of micro-organisms or suspended matters.

III. Consistency.

Normal urine is a watery fluid, pus gives it a ropy appearance.

IV. Odour.

Normal urine is described as aromatic. Diacetic acid from which acetone is developed imparts to urine a fruity odour. The odour is ammoniacal when it is decomposing.

V. Density.

The specific gravity of normal urine, which, largely depends on urea, is 1.010 to 1.020 but varies widely in health.

Estimation.

Collect urine in a large test tube and put the urinometer into it and note the specific gravity. If the quantity is too small for estimation add 2 or 4 times distilled water to it and multiply the reading according to the dilution. High specific gravity indicates concentration of urine or presence of sugar. Low specific gravity indicates excessive urine or arteriosclerotic kidney.

Chemical Examination

Reaction. Test by litmus paper.

Normal urine is generally acid due to acid salts, mainly acid sodium phosphate. Urine may be alkaline after meals, when the acid salt is replaced by di-sodium phosphates. Urine is usually more acid, before meals. Alkaline urine indicates—decomposition or cystitis.

(A)—Proteins.

Certain proteins, *e.g.*, mucin, are present in very small amounts in healthy urine.

Albuminuria:—

(*a*) Temporary or functional (tube cast absent).

(*b*) Permanent or organic (tube cast present).

Causes of temporary albuminuria:—1. Dyspepsia, functional hepatic disorders, overeating (of protein) over-exertion, nervous overstrain (anxiety, fear, etc.), general atony, anæmia; 2. drugs—turpentine, alcohol, etc; 3. temporary renal congestion, in fevers, etc.

Causes of organic albuminuria:—1. Active and passive renal congestion; 2. acute and chronic nephritis of all forms, waxy kidney, etc.; 3. syphilis, tuberculosis and gout; 4. chronic poisoning, *e.g.*, phosphorous, lead, arsenic, etc.

Qualitative examination for albumin:—

Filter the urine. If filtration does not render the urine clear, the turbidity may be due to bacteria; these may be precipitated by shaking with a little calcium carbonate when, on filtering a clear urine is obtained, which may be tested as follows:—

(1) *Heat.*—Boil some urine in a test tube, if it clears up—urates. If the reaction be acid and the urine remain clear, no albumin is present; if the urine is alkaline add acetic acid until it is acid to litmus, before boiling; opacity or precipitate indicates phosphates or albumin. Add 2 or 3 drops of acetic acid. If the urine becomes clear—phosphates; if it remains turbid, albumin is present.

(2) *Nitric Acid Test.*—Pour about 4 c.c. ot pure nitric acid into a test tube. With a pipette, allow 3 or 4 c.c. of urine to flow down the side of the tube on to the surface of the acid gently. If after half a minute a white ring forms at the junction of the liquids albumin is present; if effervescence takes place—carbonates. If no opaque white ring appears at the junction of the liquids, no albumin is present.

(3) *Trichloracetic Acid Test.*—Same as (2) only substitute saturated trichloracetic acid solution in place of nitric acid.

Quantitative estimation of albumin.

An accurate quantitative estimation of albumin may be made by means of Esbach's albuminometer. It is a thick glass tube graduated from 0 to 7 or 10.

It is important to note the specific gravity of the urine, and if this be 1.010 or more, the urine should be diluted to bring the specific gravity down to 1.008. The degree of dilution must be allowed for in the result. If the urine be alkaline it should be rendered acid in reaction by addition of a few drops of acetic acid.

Composition of Esbach's Reagent:—

Picric acid 10 grms. Citric acid 20 grm. Water 1000 c.c.

Fill the albuminometer to the mark U with urine, and add the reagent to the mark R. Mix the fluids by gently reversing the tube several times; then allow it to stand upright for 24 hours. After that period, read off, on the scale the height of the precipitate. It is important to keep the temperature constant, as the amount of precipitate varies with the temperature. Each degree of precipitate corresponds to 0.1 per cent. of albumin in the urine, as the figures on the scale represent grammes of albumin per *litre* of urine; this percentage multiplied by 4.375 = grains of almumin per fluid ounce of urine.

Example.—The precipitate is up to mark 2.

Result.—0.2 gramme in 100 c.c. or 0.2 per cent.

If the urine has been diluted, say to three times its bulk, the result should be multiplied by the degree of dilution; in this case, by three.

Note.—If the level of the coagulum be above the graduation mark 4, another sample of urine must be further diluted, and the process of estimation repeated. In an ordinary case of albuminuria, about 8 grammes, or 120 grains, of albumin are excreted daily, equal to ½ per cent. of albumin.

(B) Blood and its derivatives.

Urine containing blood appears red or smoky (brown).

Hæmaturia is the condition in which all the constituents of blood appear in urine. In *hæmoglobinuria* no blood corpuscles but only hæmoglobin is present.

(1) *Guaiac Test.*—Acidify urine if it is neutral or alkaline, pour some freshly prepared tinct. guaiac (1 in 60, not ammoniated tinct. guaiac) into test tube, and add an equal part of ozonic ether, old turpentine or hydrogen peroxide. (Absence of blue colour indicates reagent satisfactory). Then pour some urine gently on the surface of the mixture. Blue ring indicates blood.

(2) *Heller's Test.*—To 10 c.c. of urine in a tube add caustic soda till the mixture is strongly alkaline. On boiling, if blood pigment be present, a brownish red deposit of earthy phosphates will occur. The colour of the ppt. is due to the admixture of hæmatin from the blood. The supernatant liquid will become green.

(C) Bile.

Bile pigment and bile acids may occur in urine, the pigment being the more abundant. Bile gives a greenish or yellow tint to urine, and makes it less mobile.

Test for bile pigment.—

(1) *Gmelin's Test.*—If bile pigment be present, a play of colours results, from green it changes to blue, violet, red and finally yellow in the nitric acid test for albumin.

Note:—A green colour always results if bile be present. Dilution of the nitric acid with three volumes of water causes the green colour to appear more distinctly.

(2) *Smith's Test.*—Put some urine in a test tube. Pour some 10% alcoholic solution of tinct. iodine gently over it. An emerald-green layer at the junction indicates bile.

(3) *Test For Bile Salt.*—Put some flowers of sulphur on the surface of urine; they sink quickly as bile salts lower the surface tension of urine.

(D) Sugar.

Glucose is the variety of sugar most commonly found in the urine, and its occurrence is generally spoken of as "Glycosuria."

It is maintained that normal urine contains glucose but it is in amount too small to be detected by the common reagents. A positive result with any test may, therefore, be regarded as indicating abnormal condition.

Before examination for sugar, any albumin in the urine should be coagulated by boiling with acid and removed by filtration.

Examination for Sugar.

(1) *Benedict's Test*:—

Reagent.—Dissolve 173 grm. of crystallised sodium citrate and 100 grm. anhydrous sodium carbonate in 600 ccm. of water with heat. Filter and make up to 850 ccm. with water. Dissolve separately 17.3 grm. of copper sulphate in 100 c.cm. of water and make up to 150 c.cm. Mix the two solutions slowly, stirring continuously.

Test.—Add 8 drops of the sample of urine to 5 c.cm. of Benedict's reagent in a test tube boil vigourously for two minutes. On cooling, glucose will cause the appearance of a red, yellow or a green precipitate.

Benedict's test is standardised and is simpler and more reliable than the Fehling's test.

(2) *Fehling's Test*:—

Reagents.—Fehling Solution No. 1.—

Copper sulphate—34.64 grms. Distilled water—500 c.c.

Fehling Solution No. 2.—

Tartarate of sodium and potassium (rochelle salt)—173 grms.
Pot. hydrate (stick)—60 grms. Distilled water—500 c.c.

Mix equal parts of No. 1 and 2 in test tube, boil, add urine (about 1 c.c.), boil again, a reddish yellow precipitate indicates sugar, only simple discolouration does not indicate sugar. This is due to the fact that glucose has the property of reducing copper sulphate into cuprous oxide when boiled in an alkaline solution and the cuprous oxide is found as the red precipitate.

Precautions.—1. Fehling No. 1 and No. 2 are to be kept separately and are to be mixed immediately before use.

2. Albumen and phosphate give greenish-grey precipitate which interferes with the test. Hence they should be removed before testing for glucose.

3. Calcium oxalate often discolours the Fehling on boiling.

4. Chloroform used as preservative often interferes with the test. Boil the urine vigorously to drive out the chloroform before testing for glucose.

5. Creatinin and other rare substances may give confounding results.

(3) *Fermentation Test.*—Mix a little of fresh baker's yeast with about 15 c.c. of urine and fill up completely one ureometer tube or any tube closed at one end and open at the other, inverting it into a test tube containing urine; keep at room temperature for 24 hours, preferably in an incubator. Collection of gas at the top of the tube indicates glucose. This test differentiates glucose and levulose from lactose, pentose, etc., as the latter are not fermented with yeast. See that the yeast is active

Quantitative.—Dilute urine 1-10 by mixing urine 10 c.c. and water 90 c.c. Fill a burette up to mark O with this diluted urine. Mix 5 c.c. of Fehling

No. 1 and 5 c.c. of No. 2 in a porcelain dish, dilute with water, and boil. While boiling, run in the dilute urine from the burette gently till the fluid has just lost its blue colour.

The end point of the reaction can be more accurately determined by adding a drop of the fluid to a filter paper soaked in 10% pot. ferrocyanide solution acidulated with acetic acid. A brown colour indicates that all the copper has not yet been reduced.

Calculation.—10 c.c. of Fehling solution is reduced by 0.05 gramme of glucose. Thus the amount of urine required to reduce 10 c.c. of Fehling contains 0.05 gramme of glucose.

Example.—Suppose 25 c.c. of the diluted urine is required to completely precipitate the copper in 10 c.c. of Fehling's solution. The urine was diluted 1 in 10. Hence 25|10=2.5 c.c. of original urine contains 0.05 gramme of glucose. Hence 100 c.c. of original urine contains $0.05 \times 40 = 2$ grammes of glucose or 2% of sugar. For grains per ounce multiply the per cent. by 4.4 (4.375 exactly) thus the urine contains $4.4 \times 2 = 8.8$ grains of sugar per ounce.

(E) Acetone.

The odour is fruity and Fehling is reduced. Its presence usually indicates, the approach of coma in case of diabetes. Also found during fasting, anæmia, pneumonia and nephritis.

1. *Iodoform Test.*—Take urine, add few drops of Liq. potassi, warm gently, add a crystal or two of iodine and shake—urine becomes yellow or brown. Then add liq. potassi drop by drop till the colour disappears. Iodoform is precipitated and is recognised by its smell and the crystals under the microscope (hexagonal plates or stars).

2. *Rothera's test.*—Staturate a few c.c. of urine with ammon. chloride.—Add a few drops of freshly prepared concentrated solution of sodium nitroprusside, mix. Pour gently down the side of the tube strong ammonia solution—a violet ring appears at the junction.

(F) Diacetic acid.

Urine must be fresh and unboiled. Urine + 10% solution of ferric chloride = red colour, boil—colour disappears.

(G) Oxybutyric acid.

Reaction same with ferric chloride test as in diacetic acid. No other distinctive test.

(H) Indican.

This is derived from indol, a decomposition product of tryptophane of proteins. Its presence indicates putrefaction in the intestines and is increased in diseases of small intestine, and stomach with deficiency of HCl in the stomach.

Test.—Urine + equal amount of strong hydrochloric acid + a few crystals of potassium chlorate. Add chloroform and shake gently—chloroform becomes violet.

(I) Chyle.

Urine is milky, often pinkish, Filaria may be found, particularly in the night urine. Shake urine with ether—fat is dissolved and urine becomes clear.

(J) Pus.

Add liquor potassi to the deposit. If pus be present a ropy gelatinous mass is formed. Pus is best detected under the microscope.

(K) Urea.

Normal quantity passed is about 2 per cent. or 30 to 40 grammes daily.

Apparatus—Dorema's ureometer or Hind's apparatus.

Reagent.—Sodium hypobromite solution. Break a capsule of bromine (2.5 c.c.) into a vessel containing 25 c.c. of 40 per cent. of NaOH solution. Always use the reagent freshly prepared. Bromine is powerful caustic and hence is to be handled with extreme care.

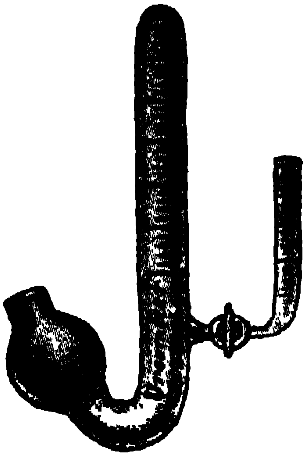


Fig. 130. Ureometer.

Method:—

1. Fill apparatus with freshly prepared hypobromite solution. (2) Fill side tube with urine up to O mark (Hind's apparatus). (3) Turn the stop-cock and run in very slowly exactly one c.c. of urine. (4) Read off the amount of gas (nitrogen) after 15 minutes, collected in the long tube.

Calculation.—If the reading be 0.02 in the side tube, it means 0.02 gramme of urea in 1 c.c. of urine or 2 grammes in 100 c.c. of urine or 2 per cent. of urea. (35.4 c.c. of gas (nitrogen) correspond to 0.1 gramme of urea).

(L) Chlorides.

Normal quantity passed in 24 hours, 10-16 grammes.

Diminished in.—Cancer, dropsy, acute fevers especially pneumonia and during rest, nephritis, starvation, etc.

Increased in.—Active exercise, diabetes.

Test.—Urine+silver nitrate solution = white flocculent precipitate.

Estimation.—1. Pour in a porcelain capsule 10 c.c. of urine + 50 c.c. of distilled water and a few drops of 1 in 20 solution of neutral potassium chromate and a pinch of calcium carbonate which neutralises any free acid present.

2. Fill a burette with standard solution of silver nitrate (29.033 grms of $AgNO_3$ to 1,000 c.c. distilled water).

3. Run the silver nitrate solution into the urine till a permanent trace of pink colour appears.

Calculation.—Deduct 1 c.c. from the number of c.c. of $AgNO_3$ solution required. Each c.c. of the remainder = 10 mgrm. of NaCl.

Total quantity of salts in urine.

This is regarded to be of more importance than the estimation of urea alone as it is indicative of functional activity of the kidneys.

It is estimated by the following methods:—

(1) *From specific gravity.*—(In the absence of sugar) doubling the last 2 figures of the specific gravity gives roughly the total quantity of solids in parts per thousand. Example.—If the specific gravity be 1018, the total solids = 36 parts per 1,000 or 3.6 per cent.

(2) *Cryoscopy.*—This depends on the fact that the freezing point of a solution of salts is proportional to the amount of salts present. If the total quantity of solids is diminished the freezing point will be correspondingly higher. *Kidney cœfficient* is the ratio of the amount of salts present in urine to the amount present in the serum and is used as a measure for renal function.

Ehrlich's diazo reaction.

Prepare two fresh solutions (1) a saturated solution of sulphanilic acid in 5 per cent. of hydrochloric acid; (2) A ½ per cent. solution of sodium nitrite.

To 5 c.c. of urine add an equal quantity of solution 1; then add a few drops of solution 2, and shake till frothy; add ammonia till alkaline. A positive result is indicated if the liquid acquires a port wine colour, and froth at the same time be red. This reaction has a significance in certain diseases.

It is most generally present in typhoid cases during the second or third week; if the case be very mild, the reaction may be absent.

It is also present in measles and in cases of tuberculosis where the disease is very active especially in the miliary form.

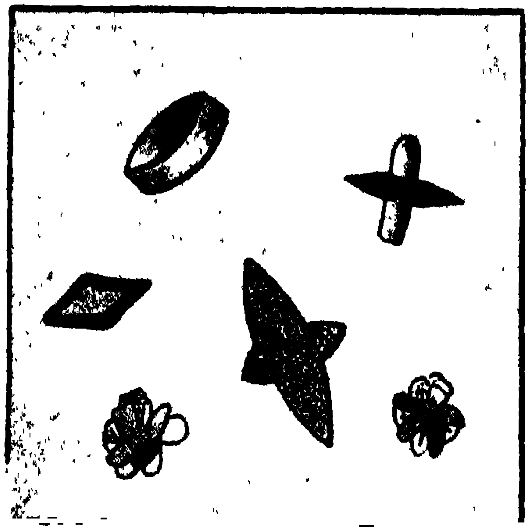


Fig. 131. Uric acid.

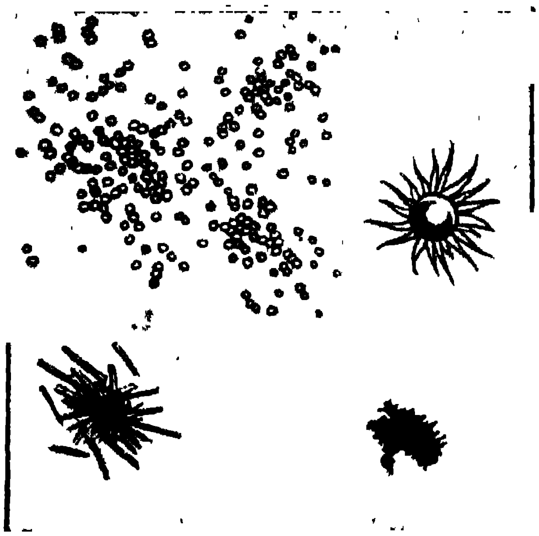


Fig. 132. Urates.

Microscopical Examination

Urine should always be examined as fresh as possible otherwise change will occur in the nature of the sediment.

For the proper examination of the sediment, too great magnification, as well as, too strong illumination, must be avoided.

The deposit should first be separated by means of a centrifuge, or by placing in a conical glass for a few hours for sedimentation. Clinically, the important deposits are those of abnormal constituents.

Unorganised Deposits.

1. *Uric acid.*—This is deposited when the urine is very acid and when there is deficiency in the pigments and salts. The deposit is yellowish or yellowish-brown crystals which under the microscope are of star, cross, lozenge or club forms. This is the only coloured crystal commonly found in urine. Daily output is 0.4 to 1 gramme.

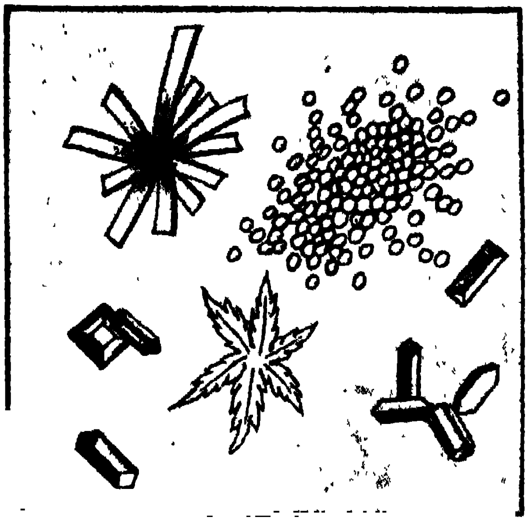


Fig. 133. Phosphates.

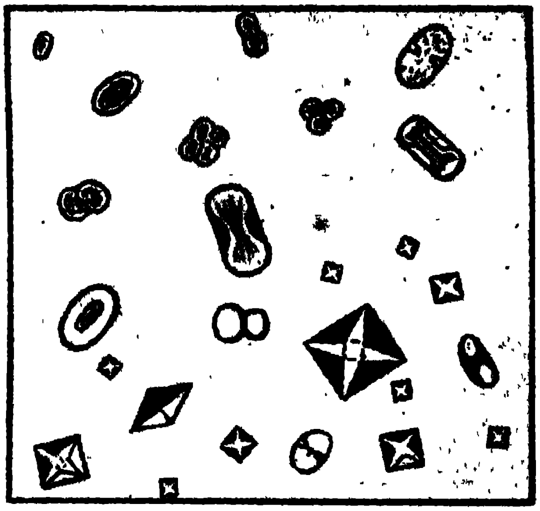


Fig. 134. Oxalates.

It is increased (*a*) *In health* with highly nitrogenous diet and after hard labour. (*b*) *In disease*—fevers, dyspepsia, gout, pernicious amæmia, etc. It is diminished in chronic gout especially just before the acute exacerbations and in chronic nephritis.

2. *Urates.*—These are mainly of sodium, potassium, ammonium or calcium and are deposited after the warm urine has become cold. The deposits are amorphous and brick coloured.

Their *constant* presence in *fresh* urine indicates *gout* or *formation of calculi* in the kidney or bladder.

3. *Phosphates.*—They occur as white flocculent deposit in feebly acid, neutral or alkaline urine. They are of the following forms:—

(*a*) Calcium phosphate—these are amorphous. (*b*) Triple phosphates—ammonium magnesium phosphate; these are present in alkaline urine in crystalline forms which are large colourless prisms, star-shaped or often feathery.

(*c*) Basic magnesium phosphate—large scattered rhombic plates.
(*d*) Neutral or dicalcium phosphate—wedge-shaped prisms arranged in stellate groups—" Stellar phosphates."

Their constant presence suggest the formation of stone.

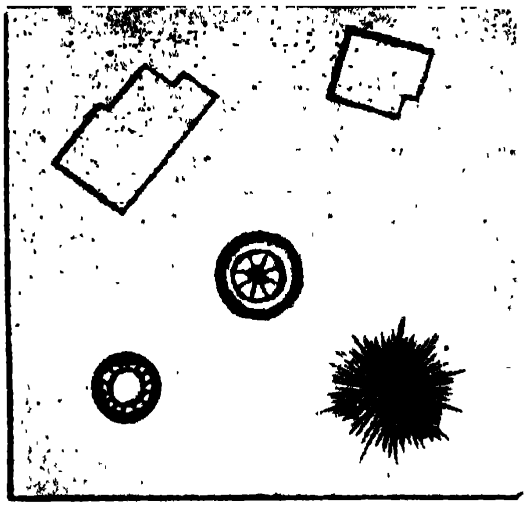


Fig. 135. Cholesterin, leucin.

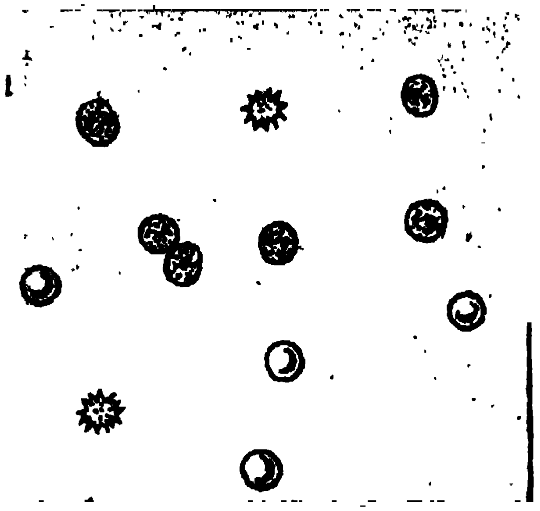


Fig. 136. Red blood cells and pus cells.

4. *Oxalates.*—Usually present in the form of calcium oxalate. They are colourless, transparent, refractile, octahedral, dumb-bell or colloidal forms. They are usually present in cases of dyspepsia and is also suggestive of formation of stone.

5. *Carbonates;* 6. *Leucin;* 7. *Tyrosin;* 8. *Cystin;* 9. *Hippuric acid.*

Table showing the common unorganised deposits in urine according to reaction:—

Colourless crystals	Calcium oxalate—dis-dissolved in HCl.	Phosphate crystals—dissolved in acetic acid.
Amorphous deposits	Urates—dissolved with heat	Amorphous phosphates—dissolved in acetic acid.
Yellow crystals ..	Uric acid—dissolved in NaOH	Ammonium biurate—dissolved in HCl.

Organised Deposits.

These are of greater importance than the unorganised forms.

Red blood corpuscles.—These are present in hæmaturia and the microscopic appearance distinguishes this condition from that of hæmoglobinuria.

Pus cells.—Are granular which clear up when treated with acetic acid and the nucleus becomes visible.

Mucus.—May be present.

Epithelium.—Squamous (vesical, or vaginal), round (renal).

Tube casts.—These may be hyaline, granular, fatty, blood, epithelial or waxy. Their presence indicates a serious pathological condition of the kidney. Blood cast indicates acute nephritis. Granular cast indicates chronic nephritis. Fatty cast indicates very chronic nephritis.

Hyaline casts are present in all forms of nephritis as well as in cases of congestion of the kidney.

Certain parasites, e.g., echinococcus and ova of bilharzia.

Spermatozoa.—It has an oval head with a long delicate tail. *Fungus.*—*Filaria sanguinis hominis.*—*Micro-organisms* (pathogenic *e.g.,* B. coli, gonococcus, tubercle bacillus) may be present.

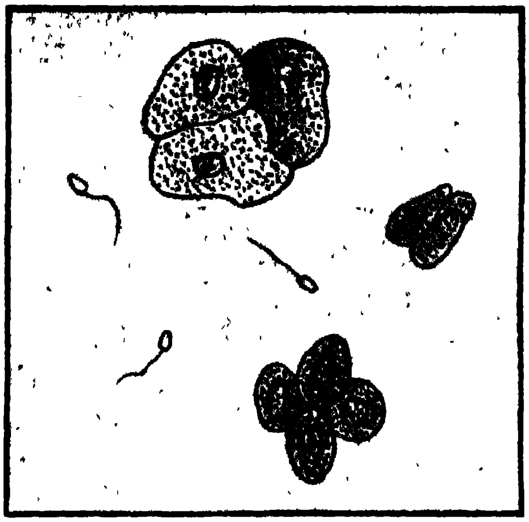


Fig. 137. Epithelium and spermatozoa.

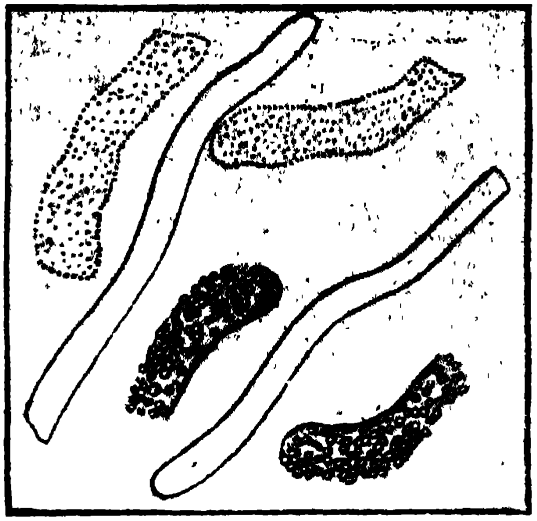


Fig. 138. Tube casts (hyaline, granular and fatty).

Bacteriological Examination

See under General Bacteriological examination.- -*Vide* infra.

EXAMINATION OF BLOOD

Enumeration of Corpuscles.

Instrument required.—Thoma-Zeiss Hæmocytometer.

A. Total red Blood cell count.

Puncture with a sharp needle the tip of a finger after cleansing the part with absolute alcohol. Slowly suck up the blood by means of the pipette (marked 0.5, 1, 101) exactly up to the mark 0.5; wipe the end of the pipette; using a bit of paper and not cloth otherwise the blood will flow out. Dip it into the diluting fluid (sodi suph. 104 grs. acetic acid 1 dr. distilled water 6 oz.) and suck up the fluid up to the mark 101, *i.e.*, a dilution of 1 in 200. Close the ends of the pipette with the thumb and the forefinger and shake thoroughly. Blow out a few drops from the pipette and then put a small droplet in the centre of the counting chamber. Put the cover slip on and see that the diluted blood is uniformly flattened out without any air bubbles or any of it flowing over the edge into the surrounding trench. If there be any overflow repeat the process. Set aside the slide for two minutes or so in order to allow the corpuscles to settle. Examine under the microscope. Focus with the low power, first, on red cells and then search for the square marks on the slide. The squares will be seen to be marked off into sets of sixteen by double ruling. Count the number of the corpuscles in 5 big squares, *i.e.*, 5 × 16 = 80 small squares. Of the corpuscles, which lie over the lines bounding the row, those, on the upper and left hand sides only, should be counted.

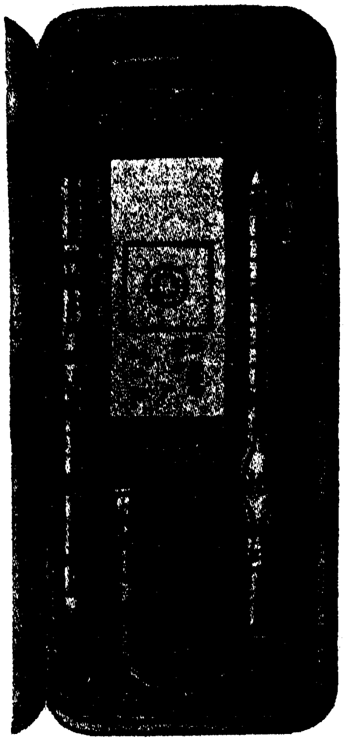


Fig. 139. Hæmocytometer.

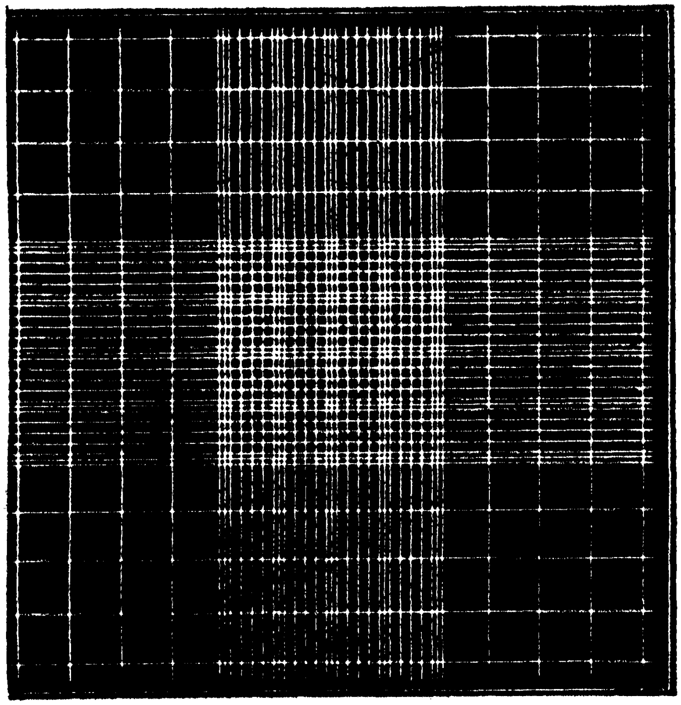


Fig. 140. Neubaur ruling for counting blood cospuscles.

Calculation.—Area of each small square is 1|20 mm. × 1|20 mm. = 1|400 sq. mm. The depth between the under surface of the cover glass and upper surface of the platform is 1|10 mm. Hence the dimension of each square is 1|400 sqr. mm. × 1|10 mm. = 1|400 cmm.

Example.—Suppose there are 480 corpuscles in the 80 small sqrs. (5 big squrs.), *i.e.*, 480 ÷ 80 = 6 in each square or 6 × 4000 = 24000 per c.mm. of diluted blood. Blood is diluted 1 in 200 hence the total number of corpuscles is 24000 × 200 = 4800000 per c.mm.

To simplify the above calculation put 0000 after the total number of the corpuscles in 5 big squares and this denotes the number of R.B.C. per c.mm.

The total number of red blood corpuscles in health is about 5,000,000 per c.mm. It is high (physiologically) in the new born child. It is increased after fasting, after removal to a high altitude and (pathologically) in (1) chronic lung diseases; (2) in concentration of blood such as diarrhœa, cholera, polyuria, rapid effusion in serous sacs; (3) in cardiac diseases, such as insufficiency or bradycardia, congenital heart diseases; (4) in severe burns; (5) after the use of iron; (6) polycythemia rubra or erythremia, and(7) acute phosphorous or carbon monoxide poisoning.

It decreases (physiologically) in menstruation, child birth, drinking of much fluid, after hæmorrhage, and (pathologically) after secondary anæmia, in leukæmia, and in pernicious anæmia.

Normal size of a red cell is about 7.5 μ in diameter.

The following terms are used to denote abnormality of red cells.

Macrocytes—size larger than normal. *Microcytes*—size smaller than normal. *Erythroblast* or *normoblast*—nucleated cell of normal size. This is normaly found in the blood of fœtus or sucklings. In adult, its presence in blood, indicates excessive demand on blood-forming organs. This is found in all forms of severe anæmia both pernicious anæmia, secondary anæmia, leukæmia, etc. *Macro or megaloblast*—nucleated cell of bigger size. *Microblast*—nucleated cells of smaller size. *Reticulocytes*—young red cells always present in blood in a proportion of from 0.1 to 1.0 per cent. of the red cells. They occur in large numbers in conditions of active blood regeneration. *Poikilocytes*—red cells of abnormal shape. *Polychromatophilia*—or *polychromasia*—red cells taking abnormal staining. *Polycythæmia*—increase in the number of red cells. *Oligocythæmia or Anæmia*—decrease in the number of red cells. *Anisocytosis*—of different sizes. *Punctate basophilia or basophile stippling*—in which certain inclusions appear in the red cell taking the blue or basic dye. They are always found in lead poisoning.

B. Total Leucocyte Count.

Suck up the blood with the other pipette (marked 0.5, 1, 11) up to the mark 0.5; wipe the end of the pipette and take up the diluting fluid (1 c.c. of glacial acetic acid, 100 c.c. of water and sufficient amount of watery solution of methylene blue to make the solution of a bluish colour) up to the mark 11 making a dilution of 1 in 20. This diluting fluid dissolves the red blood corpuscles and stains the nuclei of the leucocytes blue. Place a drop of the diluted blood in the counting chamber as before and count the corpuscles in 16 big squares, *i.e.*, all the small sqrs. in the slide 400 in number (including those with three lines).

Calculation.—Suppose there are 18 leucocytes in 400 sqrs. then $18 \div 400$ in each sqr. or $18 \div 400 \times 4000$ per cmm. of diluted blood or $18 \div 400 \times 4000 \times 20$ per cmm. of blood, *i.e.*, 3600.

Briefly, multiply the number of leucocytes by 200 and that will give approximately the number of total leucocytes.

Total number of leucocytes in health is about 5,000 to 8,000. Number exceeding 10,000 is called leucocytosis, and that below 5,000 is leucopenia.

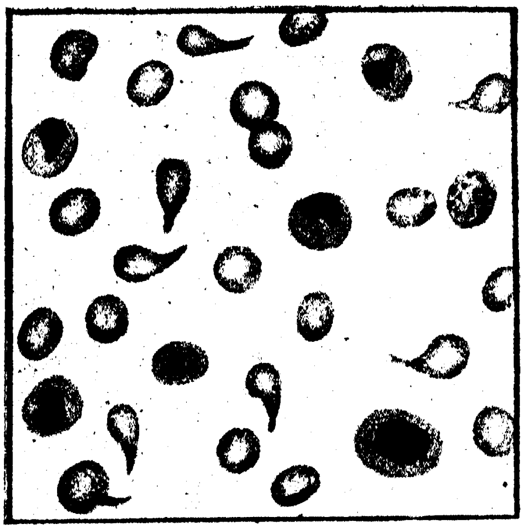


Fig. 141. Blood film of pernicious anæmia.

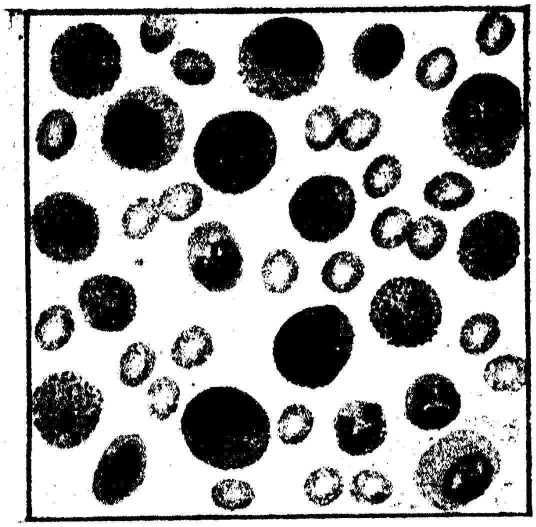


Fig. 142. Blood film of leukæmia.

Leucocytosis is found.—Physiologically, after bath, massage, breakfast, pregnancy, puerperium and in the new born, agonal (just before death). Pathologically—some acute infective conditions, after hæmorrhage, in malignant tumours, in leukæmia, etc.

Leucopenia is found in kala-azar, malaria, typhoid, dengue, measles, influenza, tuberculosis.

C. Differential Leucocyte Count.

Prepare two glass slides by thoroughly washing them in tap water; wipe away the water with a clean towel. Take a small drop of blood from the finger-prick on the slide. Place the rounded edge of the other slide over the droplet of blood, press a little sideways so that the blood spreads uniformly over the whole edge of the slide. Draw the slide at an angle of 45° over the former with gentle pressure. Dry in air and stain with Leishman's stain as follows:—

Put a few drops of Leishman's stain on the slide so as to cover it. Wait for a minute and then add double the amount of distilled water over the fluid, keep for about 10 minutes, wash off the stain in running water, dry, put a drop of cedar-wood oil on the film and examine under oil-immersion lens. The leucocytes will take a blue or violet stain whereas the red cells will take a light pink stain.

Varieties of leucocytes ordinarily present in blood.

(*a*) *Polymorphonuclear leucocytes.*—Multilobed nuclei stained violet and fine red granules in the protoplasm. They constitute 50 per cent. to 70 per cent. of the total leucocytes.

They are increased in infectious diseases, inflammatory conditions, etc., and generally represents the power of resistance of the individual.

(*b*) *Large Mononuclear Leucocytes.*—Much large cell, with a large oval nucleus, stained less violet, leaving much space in the cytoplasm. They constitute 4 to 8 per cent. of the total leucocytes.

They are increased in cholera, kala-azar and malaria.

(*c*) *Small Mononuclear Leucocytes.*—Small cell with a single round nucleus stained violet and occupying nearly the whole of cytoplasm. They form 20 per cent. to 25 per cent. of the total. They are increased in typhoid, lymphatic leukæmia, rickets, syphilis, whooping cough, tuberculosis, exophthalmic goitre, after heliotherapy, etc.

(*d*) *Eosinophiles.*—The protoplasm is packed up with coarse granules, stained red. The nucleus often shows two lobes connected by a fine strand of nuclear material. They form 2 per cent. to 4 per cent. of the total.

They are increased in skin diseases, asthma, intestinal parasitic affections, filaria and other helminthic infections, gonorrhœa, Hodgkin's disease, etc.

(*e*) *Basophiles.*—The protoplasm contains coarse, deep blue granules.

The following terms are used to denote abnormal leucocytes

(1) *Myelocytes.*—Marrow cells—they are of three varieties according to their affinity for different stains, *e.g.*, neutrophilic, eosinophilic or acidophilic and basophilic.

(2) *Myeloblasts.*—Parent cells of myelocytes, and are present in myelogenous leukæmia.

(3) *Plasma cells.*—Resembling large mononuclears with an eccentric nucleus and basophilic granular cytoplasm.

Now identify these forms of leucocytes and count about 200 to find the proportion of each of the kind present. The middle of the film should be selected and the slide to be moved forwards and backwards so as to get the average from edge to edge of the film. If the count is made along the edges of the film only, a fair average will not be otabined.

Arneth Index.

Arneth divided the polymorphonuclears under 5 classes according to the number of nuclei or nuclear fragments.

A Polymorph with one nucleus belongs to class I.
A „ „ two nuclei „ „ „ II.
A „ „ three „ „ „ „ III.
A „ „ four „ „ „ „ IV.
A „ „ five or more nuclei belongs to class V.

The sum total of classes I and II is the Arneth index.

The following are the normal figures:—

Class	I	II	III	IV	V	INDEX.
	5	35	41	17	2	40

If the index is more than 40 the picture is said to be a "drift or shift to the left;" if less than 40, "shift to the right."

A shift to the left takes place in most of the infections.

A shift to the right takes place in leprosy, syphilis, etc.

Estimation of Hæmoglobin.

Instrument required.—Sahli's Hæmometer.

Put 4 drops of dilute (1%) hydrochloric acid in the small graduated tube. Prick the finger and suck up blood into pipette up to the mark, then dip the end of the pipette in the dilute acid in the tube, blow out gently; mix thoroughly and wait for a minute. Then add the same dilute acid drop by drop till the colour of the diluted blood corresponds with the standard tint provided. The comparison should be made by holding the tubes against the light. The level at which the diluted blood stands gives the percentage of hæmoglobin. Correct the reading according to the direction given with the instrument.

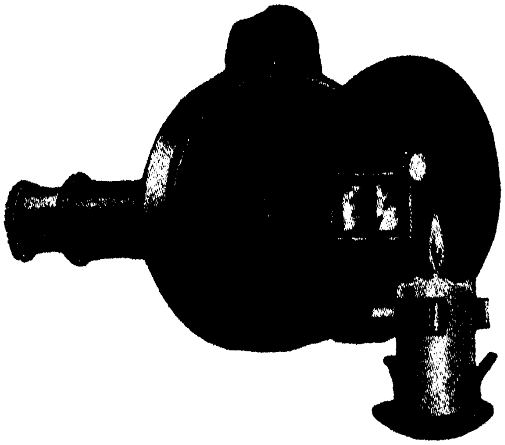


Fig. 143. Dare's hæmoglobinometer.

N.B.—The standard colour is apt to change on keeping. Dare's Hæmoglobinometer is better in this respect.

Colour index.

This is the ratio between the percentage of hæmoglobin and the percentage of the total red blood cell count (5,000,000 red cells being 100%).

Example.—Suppose red blood corpuscles = 2,000,000 and Hæmoglobin—45%.

$$\text{Percentage of R.B.C. } \frac{2{,}000{,}000 \times 100}{5{,}000{,}000} = 40. \therefore \text{ colour index} = \frac{45}{40} = 1.1$$

A simple method to get the per cent. of the R.B.C. is to double the first two figures; in this case 20×2=40.

B. Parasites in the blood.

1. Malarial parasites.

The best time, to take the blood-film, is a few hours before the chill and not during or immediately after it, as in the later case the merozoites are present in the blood, in cases of single infecion, and these forms are the most difficult ones to identify. (For details *see* under "*Laboratory Diagnosis of Malaria,*" page 227).

2. Leishman-Donovan bodies.

They are generally found in the spleen, liver, bone-marrow of cases infected by the parasites. They are also found in the blood but they are so small in number that examination of peripheral blood film often gives negative results. That they do occur in the peripheral blood is shown by the fact, that careful cultivation of the organisms in N.N.N. medium succeeds with positive finding in nearly all cases. They can be demonstrated in the peripheral blood by examination of slides containing thick leucocyte edge or thick blood films. They are always found in the polymorphonuclear or large hyaline cells. They appear as small ovoid bodies with two nuclei, one, round and big and the other, small, more or less rod-shaped, stained deep red and placed either at right angles or tangentialy to the large nucleus. There may be one or more L. D. Bodies in one cell. (*See* Coloured Plate, Fig. 1.)

3. Filaria.

They are present in blood in the stage of embryo and are termed *Microfilaria sanguinis hominis.* Blood should be taken at about midnight. Put a good sized drop of blood on a clean slide and cover it with a cover glass and make a ring of vaseline round the cover glass in order to prevent evaporation. Examine under lowpower with the diaphragm partly closed.

Another method.—Draw 20 c.mm. of blood with the pipette of Sahli's hæmoglobinometer and spread on a slide in thick film. Dry in air, put distilled water on the film to "lake" red cells. Gently pour off the water, dry and stain with either Leishman or hæmatoxylin. Dry, examine under low power and count the number of the embryos (microfilaria) in the whole film. They are beautifully stained and appear as coiled up worms.

4. Trypanosome.

This is a parasite found free in the plasma, and is elongated, and spindle-shaped with two nuclei. One, large and round,placed in the centre, is known as the macro-nucleus. The other smaller, deeply stained and placed on one end is known as the parabasal. An undulating membrane originating from the parabasal runs along the whole length of the parasite and is continued with the flagellum placed on the opposite end of the parabasal body.

It is present in the blood of sleeping sickness cases.

5. Spirochætes of the relapsing fevers.

They are cork-screw-shaped spiral parasites found free in the plasma in cases of relapsing fever.

6. Treponema pallida of syphilis.

They are spiral-shaped parasites with fine, regular numerous coils They are rarely found in the blood. Dark ground illumination is the best way to demonstrate these parasites.

C. Coagulation time of Blood.

There are two methods:—

(1) By Wright's coagulometer.—This consists of several fine capillary tubes into which blood is drawn at definite intervals and at varying intervals they are blown out. When the blood cannot be blown out it has coagulated. Note the time in each case. The difference of the time indicates the actual coagulation time of the sample of blood.

(2) By a long fine capillary tube into which the blood is drawn and the time noted. A little part of the tube is broken at intervals until a fine thread-like substance is produced in the broken part (fibrin-thread). The time of appearance of the thread is the coagulation time.

Normal coagulation time of blood is about 4 minutes. The time is diminished in kala-azar, anæmia, hæmophilia, urticaria, etc. Clotting is more rapid after meals.

Alkalinity of blood

Blood is always alkaline, normal being about N|35 to N|40. Even in extreme cases of acidosis it is never acid, the alkalinity being only diminished. The alkalinity is diminished in all cases of acidosis notably in diabetes, cholera, pregnancy, etc.

D. Bacteriological examination of blood.

See under general Bacteriological examinations.

Blood culture should be done in all cases of long-continued fever of unknown origin.

Time and quantity of blood to be taken:—

In septicæmic cases, culture should be done during the pyrexial period, specially in the afternoon when the fever is maximum. During the apyrexial period it is invariably negative.

Draw 10 c.c. of blood and distribute into four 50 c.c. broth flasks in quantity varying from 1—4 c.c.

In typhoid and paratyphoid fevers culture should be done during the first ten days. Draw 10 c.c. of blood (the later the day of illness the more blood is required) and put into 10 c.c. of 2 per cent. aqueous solution of taurocholate of soda.

Caution.—Strict asepsis is absolutely necessary. Sterilization of the syringe with absolute alcohol is never safe nor even boiling. An autoclave or hot oil is required to sterilise the syringe.

E. Biochemical Examination of Blood.

Estimation of Sugar.

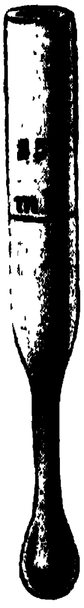


Fig. 144. Blood sugar tube.

The idea is to precipitate the proteins completely from the blood and then to estimate the quantity of sugar in the blood filtrate.

Oxalated blood is usually used. 20 mg. of potassium oxalate is quite sufficient for 10 c.c. of blood. For estimation of sugar alone, 1 c.c. or even ½ c.c. of blood is quite sufficient.

Reagents required:—

1. 10 per cent. solution of sodium tungstate. 2. $H_2 SO_4$ (2|3 N). 3. Alkaline copper sulphate solution. 4. Molybdate-phosphate solution. 5. Standard sugar solution. (0.01 per cent.). 6. Standard sugar solution (0.02 per cent.).

Method.—

(*a*) Take I volume of oxalated blood, in a 50 c.c. Erlenmeyer flask, add 7 volumes of distilled water, I volume of 10 per cent. sodium tungstate solution, and 1 volume of $\frac{2}{3}$ N-$H_2 SO_4$, drop by drop, rotate flask vigorously. The colour of the blood changes to dark brown in about 5 minutes.

(*b*) Filter carefully through a dry filter paper into a dry vessel (a large size test tube). See that the filtrate is clear.

(*c*) Take 2 c.c. of the clear blood filtrate in a Folin's blood sugar tube, add 2 c.c. of the alkaline copper sulphate solution.

(*d*) Take 2 c.c. of the standard sugar solution (0.01%) in another blood sugar tube and add 2 c.c of the alkaline copper sulphate solution.

(*e*) Immerse both these tubes in a bath of boiling water for 6 minutes.

Take the tube out, add 2 c.c. of the molybdate-phosphate sol. to each tube, dilute the contents by adding distilled water up to the mark 25 c.c. in each tube, mix thoroughly, compare the unknown with the standards in the colorimeter.

Calculation.

$$\frac{\text{weaker standard} \times 100}{\text{unknown}} \quad \text{or} \quad \frac{\text{stronger standard} \times 200}{\text{unknown}}$$

= mg. of sugar per 100 c.c. of blood.

the remaining portion of the clear filtrate can be used for the estimation of other constituents of blood.

A Simplified Bed-side Blood Sugar Method (Mukerjee's method)

In this method advantage has been taken of the new blood sugar reagents recently introduced by Folin. The sugar is oxidised with alkaline potassium ferricyanide and the ferrocyanide produced is measured colorimetrically as

Prussian blue. The colour obtained is nearly 5 times as deep as the colour obtained with the alkaline copper solutions.

The results obtained by the present method is fairly accurate for clinical purpose and the technique is so simple that it can be easily mastered in a short time. The quantity of blood necessary for the test is extremely small and can be conveniently drawn from a finger prick by means of a hæmocytometer W.B.C. pipette (up to 0.5 mark only).

Reagents.

1. *Tungstic acid reagent.*—This is prepared by diluting a mixture of 20 c.c. of 10 per cent. sodium tungstate and 20 c.c. of $\frac{2}{3}$ N sulphuric acid to a volume of 1 litre with distilled water. Add a little toluene as a preservative.

2. *Potassium ferricyanide solution.*—0.2 per cent. solution in distilled water. The solution should be kept in the dark.

3. *Sodium cyanide carbonate solution.*—Prepared by dissolving 8 grams. of anhydrous sodium carbonate and about 1.5 grm. of sodium cyanide in 500 c.c. of distilled water.

4. *Ferricsulphate solution.*—30 grm. of gum arabic is dissolved in about 600 c.c. of water by heating on the water bath. 5 grm. of ferricsulphate is dissolved separately in 75 c.c. of 85 per cent. phosphoric acid and 100 c.c. of water by means of heat. Allow the solutions to cool and then mix them together and dilute to a volume of 1 litre with distilled water.

5. *Standard glucose solution.*—0.1 per cent. solution of chemically pure glucose in saturated solution of benzoic acid in water. A little toluene may be added to it.

6. *Distilled water.*

All these reagents if kept properly have good keeping qualities. They can be easily prepared in any chemical laboratory and stocked for later use.

Apparatus.

(1) Four test tubes. (2) Two 25 c.c. graduated cylinders (of the same size and make); (3) One mark capillary pipette with attached rubber tubing and mouth piece 1; (The pipette in Sahli's hæmoglobinometer or a hæmocytometer W.B.C. pipette will do.) (4) An aluminium vessel or an ordinary glass beaker to serve as a water bath; (5) One spirit lamp with tripod stand; (6) Strips of filter paper.

Estimation of blood sugar

With the pipette draw the standard sugar solution (0.1 per cent.) up to mark. (If the W.B.C. pipette of a hæmocytometer is used it is quite sufficient to draw up to the 0.5 mark). Wipe off the outside of the pipette with a piece of filter paper. Blow out the sugar solution completely on a small piece of filter paper. Put this piece of filter paper in a test tube marked 'S'. It is not necessary to wash the pipette after this. With the same pipette draw blood up to the same mark in the usual way from a finger prick. Wipe off the blood on the outside of the pipette with a piece of filter paper. Then blow out the blood completely on another small piece of filter paper. Put this piece of filter paper in a test tube marked 'U.' We have now exactly the same volumes of the standard sugar solution and of blood in the two tubes 'S' and 'U.' If the pipette of Sahli's hæmoglobinometer is used this volume is only 20 cmm. (*i.e.*, 1-50th of a c.c.).

Put the two test tubes 'U' and 'S' in the boiling water bath for 2 minutes. Then with a graduated cylinder add about 5 c.c. of the tungstic acid reagent to each of the test tubes. Allow to remain in the boiling water bath for 5 minutes. Then take them out and by means of a glass rod or a clean piece of wire remove the pieces of filter paper. Care is taken to remove as little of the liquid as possible with the filter paper. To each of the test tubes then add with a graduated cylinder 1 c.c. of the potassium ferricyanide solution and 1 c.c. of the carbonate solution. Put the test tubes in the boiling water bath again and allow to remain for 7 to 8 minutes. Then remove and cool. Add 3 c.c. of the ferricsulphate solution to each of the test tubes. Mix by gentle shaking and let stand for 5 minutes when the colour of Prussian blue develops.

Pour the contents of the test tube marked 'S' into one of the graduated cylinders and mark it 'S.' Similarly pour the contents of 'U' into the other cylinder and mark it 'U.' Dilute both 'S' and 'U' up to the 10 c.c. mark by adding a few drops of water if necessary. The intensity of colour in each cylinder is proportional to the amount of sugar present.

Calculation.—The sugar is determined colorimetrically by what is known as a "test tube colorimeter"—a well known procedure when a regular colorimeter is not available. If the colour of the solutions in 'S' and 'U' match each other exactly when made up to the same volume, then 'U' contains the same amount of sugar as the standard 'S,' *i.e.*, the blood sugar in this particular case is 0.1 per cent. If the colour of 'U' is deeper than that of 'S' then go on diluting 'U' by adding water drop by drop carefully till the colour of 'S' and 'U' match correctly. The blood sugar is given by the following formula:—

$$\text{Blood sugar (in \%)} = \frac{\text{X (dilution or total vol. of sol. in U)}}{\text{10 (dilution or total vol. of sol. in S)}} \times 0.1\%.$$

Example.—If 'U' had to be diluted up to the 22 c.c. mark to match correctly with 'S' (Vol. — 10 c.c.) then blood sugar = 22|10×0.1% = 0.22%.

In diabetics having blood sugar contents higher than 0.25 per cent., *i.e.*, when the colour of 'U' even after diluting up to the 25 c.c. mark is still deeper than that of 'S' proceed as follows. Dilute 'U' up to the 25 c.c. mark then discard half of the liquid (12.5 c.c.) and again go on diluting till exact colour match with 'S' results. In this way the unknown 'U' can be diluted 2 × 2.5 times and we can estimate a blood sugar content up to 0.5 per cent. For still higher blood sugar contents the unknown should be diluted even further.

In normal individuals and in hypoglycemia when the sugar content of 'U' will be less than 0.1 per cent. go on diluting 'S' till the colour of 'S' matches exactly with that of 'U,'—'U' being kept at the original volume of 10 c.c. The blood sugar is given by the following formula:—

$$\text{Blood sugar (in per cent.)} = \frac{\text{10 (dilution in 'U')}}{\text{X (dilution in 'S')}} \times 0.1 \text{ per cent.}$$

Example.—'S' had to be diluted to the 20 c.c. mark to match correctly with 'U' (Vol. 10 c.c.) then blood sugar = 10|20×0.1%=0.05%.

The advantages of this method are:—

(1) No special apparatus is necessary.

(2) A very small amount of blood (about 1|50 of a c.c.) from a simple finger prick is sufficient.

Milking or squeezing the finger for blood is not only unnecessary but should be avoided as the blood becomes mixed with tissue-fluids.

(3) The collection of the blood with the capillary pipette is extremely simple and familiar to most practitioners. A W. B. C. pipette can very well be used for the purpose.

(4) The manipulations are quite simple and can be mastered easily.

(5) The results obtained are quite accurate for clinical work.

The preparation of protein-free filtrate.

One volume of oxalated blood is mixed with 7 volumes of distilled water, one volume of $\frac{2}{3}$N H_2SO_4 and one volume of 1 per cent. solution of sodium tungstate in a glass flask. The flask is then thoroughly shaken and let stand for 5 minutes, It is then filtered. The resulting clear filtrate is then analysed for N.P.N. and urea content.

5 c.c. of this filtrate are used for each of these estimations.

Estimation of non-protein nitrogen.

In a hard-glass test tube introduce the following:—

(*a*) 5 c.c. of filtrate

(*b*) About 0.5 c.c. of digestion mixture, which is composed of

Copper sulphate (5% sol.) 50 c.c. H_2SO_4 (pure) 100 c.c. Phosphoric acid (85%) 300 c.c.

Heat over a microburner, continue the heating until the solution is clear, allow it to cool, add about 10 c.c. of distilled water and then immerse into a boiling water bath. Filter while still hot, in a 50 c.c. measuring flask and extract with 10 c.c. water similarly and cool the contents of the flask.

To a 100 c.c. measuring flask, add 3 c.c. of standard ammonium sulphate solution, 1 c.c. of digestion mixture and add 60 c.c. of water.

Estimation of blood urea.

Into a hard-glass test tube put 5 c.c. of filtrate, 2 drops of buffer solution and one piece of urease paper. Incubate in a water bath at 55°C for 10 minutes. After that period remove from the bath and add 2 c.c. of saturated borax solution. Insert a spiral ring of wire. Close mouth of test tube with a rubber stopper fitted up with a bent delivery tube dipping into a test tube (graduated at 25 c.c.) containing 2 c.c. of 0.05 N acid.

The test tube containing the blood filtrate is heated until the content begins to boil. The distillation is continued until the content of the test tube containing the 0.05 N acid is nearly doubled. The delivery tube is then removed from this latter test tube and the distillation stopped. The distillate is then cooled and the volume made upto 22 c.c. with distilled water.

3 c.c. of the standard ammon. sulph. solution are measured into a 100 c.c. measuring flask and about 80 c.c. of water added to the same.

10 c.c. of Nessler's solution added to the standard and 2.5 c.c. to the tube containing the distillate. The volumes are made upto 100 c.c. and 25 c.c. respectively. Then compare in the colorimeter as in the estimation of N. P. N.

Nessler's solution is composed of Pot. iodide 150 grm. Iodine 110 grm. Mercury (metallic) 140 gram. Water 100 c.c.

For its preparation Folin's "Laboratory Manual of Biological Chemistry" should be consulted.

Buffer solution.—[For use in blood urea determination.]

69 grm. monosodium phosphate.
179 grm. crystallised disodium phosphate. 1 litre distilled water.

Urease paper.

Thirty grm. of powdered soyabean are ground up with 10 grm. of permutit powder. The whole is well shaken with 120 c.c. of 16 per cent. alcohol. The resulting fluid is filtered and strips of filter paper are drawn through the filtrate, after which they are hung up to dry. A piece of dry paper about 1 inch square is sufficient for one determination of blood urea.

Standard solution of Ammonium sulphate contains 1 mg. of nitrogen per 10 c.c. (*i.e.,* 0.4716 gm. of pure ammon. sulph. per litre).

Interpretation.—

Normal urea content of the blood is 20 to 40 mgm. per 100 c.cm. Increase of blood-urea indicates renal inadequacy and a figure of 100 mgm. per 100 c.c. or more is of serious import.

Extrarenal conditions of the rise of blood-urea content depends on inadequate supply of water for the excretion of urea *e.g.* in cholera, gastro-enteritis, diabetic coma, intestinal obstruction etc.

BLOOD CHEMISTRY IN CLINICAL MEDICINE

The application of the study of blood chemistry to clinical medicine is of quite a recent development. This has only been possible with the development of micromethods of analysis by which minute quantities of material can be accurately estimated.

The clinical value of blood chemistry has been recognised in three main pathological conditions:

(1) Diabetes and glycosurias.

(2) Renal disease.

(3) Acidosis and alkalosis.—All those conditions in which there is tendency towards a change in the reaction of the blood.

Besides these three conditions a study of blood chemistry is also of importance in some other conditions such as tetany, rickets, gout, toxæmias of pregnancy, etc. Moreover new and clearer conceptions regarding the nature of many diseases are being evolved by a study of blood chemistry.

Diabetes and glycosurias.

A typical case of diabetes is characterised by polyuria, thirst and hunger. The glycosuria is often accompanied by the presence of "*Ketone bodies*"—acetone, diacetic acid and *B*-Oxy-butyric acid in the urine. The malady generally tends to develop into fatal coma.

Apart from this typical case there are cases of all shades of severity,—from a case with only the symptom of slight glycosuria to one of the severest type, with the most marked symptoms. The question naturally arises: When a patient has glycosuria and no other symptoms, should he be considered to be a diabetic? This question can be answered at once by an examination of the sugar content of the blood.

Moreover as diabetes is mainly a disease of carbohydrate metabolism the estimation of blood sugar is of very great importance as regards the progress and treatment of the disease. With the introduction of insulin treament the blood sugar estimation in diabetes has become absolutely essential as careless use of insulin without reference to the sugar content of the blood is extremely dangerous.

The *Acidosis* in diabetes is due to the presence of abnormal amounts of acids in the body. These acids are chiefly: *B*-oxy-butyric and diacetic acids. The tendency for the development of coma which is so marked in diabetes is intimately related to this accumulation of organic acids in the body. Now if we could estimate the amount of retained acids in the body we will be able to fore-cast with some exactitude the chances of coma developing. Direct estimation being impossible we have to rely on certain changes in the blood and urine. The extent of these changes indicates the condition of the patient and the chances of coma developing. The chief methods available for the investigation of acidosis are:—

1. Determination of changes in the reaction of the blood.
2. Estimation of the dissolved carbonic acid of the blood.
3. Estimation of the CO_2 tension in the alveolar air.
4. Estimation of the sodium bi-carbonate of the blood.

To understand the significance of these investigations, it is necessary to consider the changes which excess of acids in the body produces in the blood.

The reaction of the blood is mainly dependent on the relationship of two variables, the carbonic acid and the sodium bi-carbonate present in blood. Any fall in the carbonic acid content, the bi-carbonate remaining constant produces a rise in the pH *—a shift of the reaction towards the alkaline side—while any diminution in bi-carbonate content, carbonic acid remaining constant, will shift the reaction towards the acid side.

With a pH of 7.4 (normal) the ratio $\frac{H_2CO_3}{NaHCO_3}$ is about equal to 1|20. So long as this relationship is maintained the reaction remains constant. It is essential to realise here that it is not the absolute amount of H_2CO_3 or $NaHCO_3$ present which is of importance but the proportion which these two variables bear to one another. The pH of blood will remain normal with a high, low or normal bi-carbonate content as long as equivalent changes occur in the H_2CO_3.

The relationship $\frac{H_2CO_3}{NaHCO_3} = \frac{1}{20}$ is maintained with extraordinary constancy, for whenever a little too much carbonic acid accumulates in the system the respiratory centre is stimulated and the excess of CO_2 is thrown out. Any tendency to excess of bi-carbonate is also dealt with by various ways. When an acid is added to the blood there is a reduction of the bi-carbonate content. This results in a temporary increase in CO_2 which is thrown out by increased ventilation of the lungs so as to maintain the necessary constant relationship. The blood now contains the two substances in the same relative proportion but the total amount of *both* is reduced. The extent to which these bodies are reduced indicates the degree of the acidosis. Now as the amount of CO_2 in the alveolar air depends directly on the amount in the blood the alveolar air of the patient with little low carbonic acid content in the blood will have less CO_2 than is present in normal alveolar air. Thus in acidosis there is a decrease of bicarbonate acid in blood and a consequent lowering of the CO_2 tension in the alveolar air and a measurement of this decrease is an index of the extent of the acidosis.

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* The reaction of a solution is acid when it contains an excess of hydrogen ions (H) over hydroxyl ions (OH). The reaction is alkaline when the hydroxyl ions predominate over hydrogen ions. It is neutral when the hydrogen and hydroxyl ions exist in equal number. A *normal* acid contains one gramme of hydrogen ion in one litre and a *normal* alkali contains the *equivalent* amount of hydroxyl ions in one litre of the solution.

Pure water dissociates to form hydrogen and hydroxyl ions. The amount of hydrogen ion present in a litre of pure water at 20°C is approximately 1-1,00,00,000 gramme. It also contains an equivalent amount of hydroxyl ions. So we may consider pure water to be 1|1,00,00,000 N acid and also 1|1,00,00,000 N alkaline. To avoid using large figures it is convenient to write 1|1,00,00,000 N as 10^{-7} N. It is still more convenient, as suggested by Sorensen, to use the logarithmic notation, dropping the minus sign and say pH7.

The higher the pH the more alkaline (or less acid) is the solution.

pH7 = neutral (pure water).

pH—more than 7 = alkaline, pH—less than 7 = acid.

Renal disease.

One of the chief functions of the kidney is the removal of waste products from the body. So of all renal tests the most important in acute or chronic disease, is the estimation of the retained nitrogenous bodies in the blood. The chief of these nitrogenous bodies are urea, creatinin, uric acid and purin bodies. All these substances together comprise the " non-protein nitrogen."

The estimation of blood urea is of great help in acute and subacute cases of nephritis specially in advanced cases of arteriosclerotic kidney. Estimations of blood urea at intervals of a week or so give valuable information. Whatever be the clinical condition a steady, persistent rise in blood urea indicate a bad prognosis, on the other hand, however severe be the symptoms if the blood urea comes down steadily the outlook is good.

Essential hypertonia without any kidney involvement can thus be separated from a case of high pressure where the kidneys are affected as a part of general arteriosclerosis. Again, cases of hypertonia ultimately may have myocardial trouble, and this stage can be differentiated from cardiac decompensation, pure and simple by noting the amount of " Nitrogen retention." Gastric symptoms in a case of nephritis can be diagnosed early from a blood picture, at a stage when even the urine test may give no clue to the underlying condition.

Value of over 4 milligrams of creatinin per 100 c.c. of whole blood generally indicates serious impairment of the renal function. The amount of chloride and nitrogen retention gives valuable guide to diet. A high uric acid finding in the blood alone is characteristic of gout and can thus be diagnosed from simple rheumatic fever and other arthritis (in which if there be uric acid retention, other nitrogenous bodies are retained also).

Acidosis and alkalosis.

Clinically acidosis may be considered under three heads:—

1. Acidosis resulting from excessive formation of diacetic acid and oxybutyric acid, *e.g.*, in diabetes.
2. Acidosis due to failure of phosphate excretion—retention of acids in the system—*e.g.*, in nephritic acidosis.

3. Acidosis due to failure to remove carbonic acid, *e.g.*, in uncompensated heart disease.

It is well known that a surgeon is particularly anxious when he has to operate on a case of diabetes, because he is afraid of bringing on acidosis. This anxiety is due to the fact that anæsthetics, chloroform and ether reduce the CO_2 carrying capacity of the plasma. Hæmorrhage and shock enhance the danger. The vomiting that follows administration of anæsthetics, in certain patients is also due to the acidosis produced as a combined effect of the anæsthetics and the operative procedure.

Alkalosis is a rare condition. It is found associated with tetany and certain gastric disorders.

SEROLOGICAL EXAMINATION OF BLOOD

Widal Reaction.

This test is based on the fact that in almost every case of typhoid fever after the first few days (9 days) a substance is formed in the blood serum of the patient which causes the typhoid bacilli from a broth culture to collect in "clumps." This phenomenon is called "*Agglutination.*"

There are two methods—the macroscopic and the microscopic. The macroscopic method is now-a-days more extensively used than the microscopic one. The more important reasons being—(1) the end-point of agglutination more accurately obtained (2) the culture is a standard one and so the results of works done in different parts of the world may be comparable (3) does not require well equipped bacteriological laboratory and the microscope, hence can be used even by the village practitioner (4), no chance of infection of the laboratory worker as dead organisms are used.

The Widal reaction is usually done against B. typhosus, B. paratyphosus, B. enteritidis Gaertner and cholera vibrio.

The macroscopic method.

Take 2 c.c. of blood from the vein of the patient or a Widal tube full of blood from the finger tip and let it clot. Collect serum with a pipette. Dilute 2 drops of serum with 18 drops of normal saline. With a standard pipette measure out the reagent as follows:—

Number of tube.	Drops of saline solution.	Drops of serum diluted 1 in 10.	Drops of emulsion standard.	Final dilution.
1	0	10	15	1 in 25
2	5	5	15	1 in 50
3	8	2	15	1 in 125
4	9	1	15	1 in 250
5	10	0	15	control.

The emulsion used in this reaction is a formalinised broth culture of typhoid bacillus containing about 250 million organisms per c.c. This emulsion of dead organism can be used for several months.

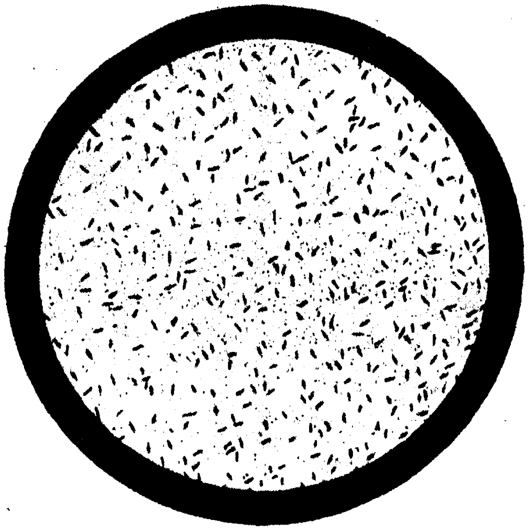


Fig. 145. B. typhosus before agglutination.

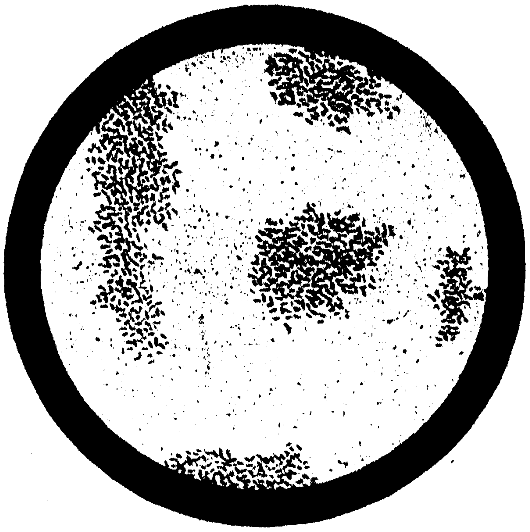


Fig. 146. B. typhosus after agglutination.

Keep the tubes for 2 hours in a bath at 55°C. Take out and wait for 15 minutes. A thermos flask can take the place of a bath, where the latter is not available. Note agglutination at the end of that time. Agglutination in the second tube upwards is diagnostic of typhoid.

The microscopic method.

Method of collecting blood—Sterilize the finger of the patient, prick with a needle, squeeze out a large drop of blood. Break off the ends of the Widal tube and put one end of the tube in the drop of blood on the finger the other end of the tube being on a lower level. Blood will enter the bulb by capillary action. When enough has been collected, seal both the ends of the tube over the flame and keep aside for the serum to separate.

Technique of the test. *Things required*—

(1) One watch glass for saline and another for broth culture of B. typhosus; (2) Clean slides and cover glasses; (3) Wright's pipette—a long fine capillary tube with a bulb on one end, to which a rubber teat is attached; (4) Young broth culture of B. typhosus. (24 hour's growth); (5) Sterile normal saline.

Take the capillary tube (Wright's), and break off both the ends, attach the rubber teat to the end near the bulb. Make a mark about one inch from the other end. Break off the straight end of the Widal capsule containing the blood of the patient and dip the marked end of the Wright's tube through the broken end into the blood serum which has by this time separated out.

Draw out the serum up to the mark on the capillary tube by relaxing the rubber teat, allow a little air to enter the tube and then dip the end into saline and draw it again up to the mark, again allow a little air to enter. Repeat the process until 9 parts of saline have been drawn up. Press the rubber teat and transfer the whole contents on a slide; mix up thoroughly. Thus the serum is diluted 1 in 10. Take one part of this 1 in 10 serum and one part of broth culture of B. typhosus and place on another slide. The serum in this mixture is diluted in 1-20. Put a cover glass on.

To make a solution of 1 in 40, take one part of 1 in 10 serum, one part of saline and two parts of broth culture.

To make a dilution of 1 in 100, take one part of 1 in 10 serum, four parts of saline and five parts of broth culture.

Put the dilutions 1 in 20, 1 in 40, 1 in 100 separately on a slide under cover glasses. Remove traces of serum from the capillary tube by repeated washing with saline. Take one part of broth culture and one part of saline, mix, put on another slide under cover glass as a control.

Keep these two slides in the incubator for half an hour, and then examine under microscope. Note in which dilution the bacilli are clumped. The reaction is positive when all the organisms have collected in clumps with loss of motility. It is partially positive when there is a tendency to form clumps but still some amount of motility is present. The result is negative when there is no tendency to clump. The *control* should always be negative.

Interpretation of the findings.

1. In apparently normal individual—the reaction is usually negative or give a very low agglutination titre (1 in 20) but a very small percentage of them (about 4%) give a positive reaction (1 in 40 or even 1 in 80). It is believed that some of these people are carriers.

2. Patients suffering from the typhoid or allied infection show little or no agglutinins in the blood during the first week. From the tenth day the agglutinins increase rapidly in intensity, reaching the maximum at the end of the third week and then falls gradually over months or even years. The highest titre of agglutination may reach 1 in 40,000.

3. After protective inoculation with a vaccine prepared from typhoid, paratyphoid bacilli (T. A. B. vaccine). The agglutinins reach their maximum after 2—3 weeks at which level they remain for years with very little fluctuation unless the patient becomes infected with the enteric groups of organisms.

Determination of the opsonic index (Dreyer's method).

Things required:—

(1) Serum of the patient. (2) Serum of a healthy person.

(3) Bacterial emulsion with normal saline from young agar culture.

Put 1 c.c. of blood from a healthy individual into 10 c.c. citrated saline, mix, centrifuge, withdraw the supernatant fluid with a pipette and add fresh saline and again centrifuge. Repeat the whole thing three or four times. The sediment will consist of red cells and leucocytes. Pipette off the upper buffy layer of leucocytes and place on a watch glass.

Technique.—Take one capillary pipette and mark about one inch from the end. Draw in one unit of the leucocyte emulsion by relieving pressure from the rubber teat, also one unit each of the bacterial emulsion and the patient's serum. Transfer to a slide, mix thoroughly, take again into the pipette and seal the end. Take another pipette, proceed as before and take in one unit each of the leucocyte emulsion, bacterial emulsion, and normal serum, mix, draw up into the pipette and seal the end. Keep the two pipettes in the incubator for half an hour. Take them out of the incubator, break the ends, transfer the contents on one slide each, and make a smear from each of them. Stain with Leishman's stain or methylene blue and count the number of bacteria ingested by 100 leucocytes in each case.

Opsonic Index.—This is the ratio between the number of bacteria ingested by a definite number of leucocytes with patient's serum and the number of organisms ingested by the same number of leucocytes with normal serum.

Example.—Suppose the smear from the first pipette gives 600 organisms in 100 leucocytes and smear from the second pipette 1,000 organisms in 100 leucocytes.

Then the opsonic index $(\mu) = \frac{600}{1000} = \frac{6}{10} = 0.6$.

Wassermann Reaction

This is a practical application of the complement-fixation or the complement deviation phenomenon and is used for the purpose of diagnosis of syphilis from the patient's blood.

The requirements for the test are as follows:—

1. Serum of the patient to be tested. 2. Complement—fresh serum from a guinea-pig. 3. Antigen—alcoholic extract from normal beef-heart. Cholesterin may be added. 4. Suspension of sensitised red blood cells—washed red blood cells from sheep's blood and immune serum from a rabbit allowed to act together for half an hour. 5. Normal saline (0.85%). 6. Water bath, pipettes, watch-glasses etc.

The first three items are incubated together and then the fourth item which is the indicator (hæmolytic system) is added, to test for free complement.

Preparation of the reagents.

1. Patient's serum.—After the clear serum has separated off from the blood, the complement present in it must be destroyed by heating it in water bath at 55°C. for ten minutes.

2. Complement.—This is present in every fresh serum. It is the common practice to use blood of a guinea-pig. The blood is obtained from the heart of the animal. Safest amount is 3-5 c.c.

3. Antigen.—The left ventricle of beef-heart is taken and is carefully separated from fat. It is minced and ground with sand

in a morter and mixed with absolute alcohol (1 grm. of heart to 9 ccm. of alcohol). The mixture is shaken for half an hour and filtered. 1% alcoholic solution of cholesterin is added in the proportion of 2 parts of cholesterin solution to 3 parts of heart extract. The extract and the cholesterin solution keep well for about 6 months when kept separately. They are mixed just before use.

4. The hæmolytic system.—This is a suspension of washed sheep's corpuscles sensitised by the addition of the heated serum of a rabbit which has been immunised against those red cells. The blood is drawn from the jugular vein of a sheep in equal quantity of citrated saline and thoroughly mixed to prevent coagulation. It is then centrifuged and the corpuscles are washed repeatedly for 3 times with normal saline to get cells free from all serum. This constitutes washed red blood cells. To immunise a rabbit against these cells, the animal is injected into the ear vein with these cells in doses of 1 c.c., 1.5 c.c., 2 c.c., and so on at intervals of 4 days until the serum of the animal hæmolises the red blood cells in proper strength. The animal is then bled to death and the serum collected and inactivated at 55°C. It is then preserved in ice chest.

Standardisation of the reagents.—

In Harrison's method the measurements are expressed in volumes of varying dilutions. For the sake of convenience 0.12 c.c. is taken as one volume.

Titration of Hæmolytic amboceptor.

This depends in ascertaining the minimum amount of rabbit's serum which is sufficient to hæmolise completely a volume of sheep's corpuscles in presence of the complement. The anti-sheep serum is diluted in proportion of 1 in 125, 250, 500, 750, 1,000, 1,500, 2,000 and so on. One volume of 3% suspension of corpuscles is taken in different tubes and to each one volume of the different strength of diluted anti-sheep serum is added and incubated at 37°C. for half an hour after which one volume of 1 in 10 dilution of complement is added to each tube; one volume of saline is added to each tube to make the bulk of the fluid equal to 4 volumes. The tube which contains the smallest amount of amboceptor with complete hæmolysis is sought. This is the ***minimum hæmolytic dose*** (*M.H.D.*). An amboceptor of lower titre than 1 in 1,000 should not be used. In the test proper 5 M.H.D. of hæmolytic amboceptor is used. Amboceptor is stable and needs titration every three months. It is well to allow the washed cells to remain in contact with amboceptor for half an hour before use.

Titration of the Complement.

The complement is to be titrated every time before use. It is diluted in proportion to 1 in 10, 20, 30, 40, 60, 80, 100. The titration is done in the similar way as the amboceptor. To each tube one volume of the sensitised cells, one volume of varying strength of the complement and two volumes of normal saline are added and incubated. The smallest amount of complement which causes complete hæmolysis is the M.H.D. of the complement. In actual test 3 M.H.D. is used.

Titration of the antigen.

The extract may vary in its deviating power according to the manner in which it is diluted. With heart-cholesterin extract it is best to dilute quickly. Before titrating the extract, it is advisable to ascertain that it is not unduly hæmolytic. To do this one volume of the 1 in 5 dil. of the extract should be incubated with one volume of the suspension of cells and 2 volumes of saline for an hour. If no hæmolysis is apparent at the end of this time, the extract is satisfactory. The extract is diluted in proportion of 1 in 5, 7.5, 10, 15, 20, 40, 60, 90—200.

Four rows of test tubes are set out and are marked A B C D respectively. The row A contains serum (1 in 5 dil.) of a known negative case; row B contains serum of known strongly positive case. C contains serum of weakly positive case and D contains serum of patient suffering from psoriasis etc. Into each tube 3 M.H.D. of complement added. Each row contains one volume of the extract of the varying dilution.

After incubating for half an hour at room temperature and another half an hour at 37°C. one volume of the sensitised cells is added to each tube and incubation is continued for another half an hour. The tubes are then carefully examined.

The tubes in row A will show complete hæmolysis, in row B there will be no hæmolysis (complement fixed) except in the tubes containing highly diluted extract (1 in 200 etc.). In row C hæmolysis may be partial in the tubes containing 1 in 15, 20 dil. extract, but is complete in tubes containing higher dilution of the extract. In row D there may be slight evidence of complement fixation (no hæmolysis) in the tubes containing 1 in 5, 7.5, or 10 but none in 1 in 15 tube.

The strength of the extract to be used in the test proper is provisionally fixed at half the amount which just fails to fix 3 M.H.D. complement when in contact with normal serum.

The test proper

One known positive serum and one known negative serum should be tested with the sera under examination as controls.

A rack containing 4 parallel rows of holes is taken. The first three rows are filled with empty tubes, the back row containing 1 in 5 dil. sera (inactivated) properly marked 1 2 3 4, so that each serum has 3 empty tubes in front. Each sample of diluted serum is put in the 3 empty tubes in front, one volume in each. When all the samples have been dealt with in this way one volume of the diluted antigen is put into the first two rows; the third row tubes receive no antigen. One volume of saline is added in the third row to make the bulk of the fluid same in all the tubes.

The complement is then added. The tubes in the first and third rows receive 3 M.H.D. while those of the second row receives 5 M.H.D. The tubes are shaken and allowed to stand at room temperature for half an hour, then in water bath (37°C.) for another half an hour. To each tube is, then, added one volume of the sensitised corpuscles and incubation allowed to proceed. The tubes are shaken every five to ten minutes. The known negative serum tubes are examined for complete hæmolysis. As a rule, the reading is taken half an hour after the cells are added. All the tubes in the third row will show complete hæmolysis while the tubes of the first and the second row containing the positive serum will show no hæmolysis.

The reading is indicated as follows:—

++	When both the tubes of the first and the second rows will show failure of hæmolysis.
+	When the tubes of the first row show hæmolysis but the second row tubes show failure of hæmolysis.
±	When the first row of tubes shows partial hæmolysis while the second row shows failure of hæmolysis.
—	When the tubes in both the first and second rows show complete hæmolysis.

Kahn's Precipitation Test.

Practically all alcoholic extracts of animal tissue, no matter how prepared, when properly mixed with syphilitic sera, will produce precipitates after sufficient incubation. This, it would appear, might render the evolvement of a precipitation test for syphilis compara-

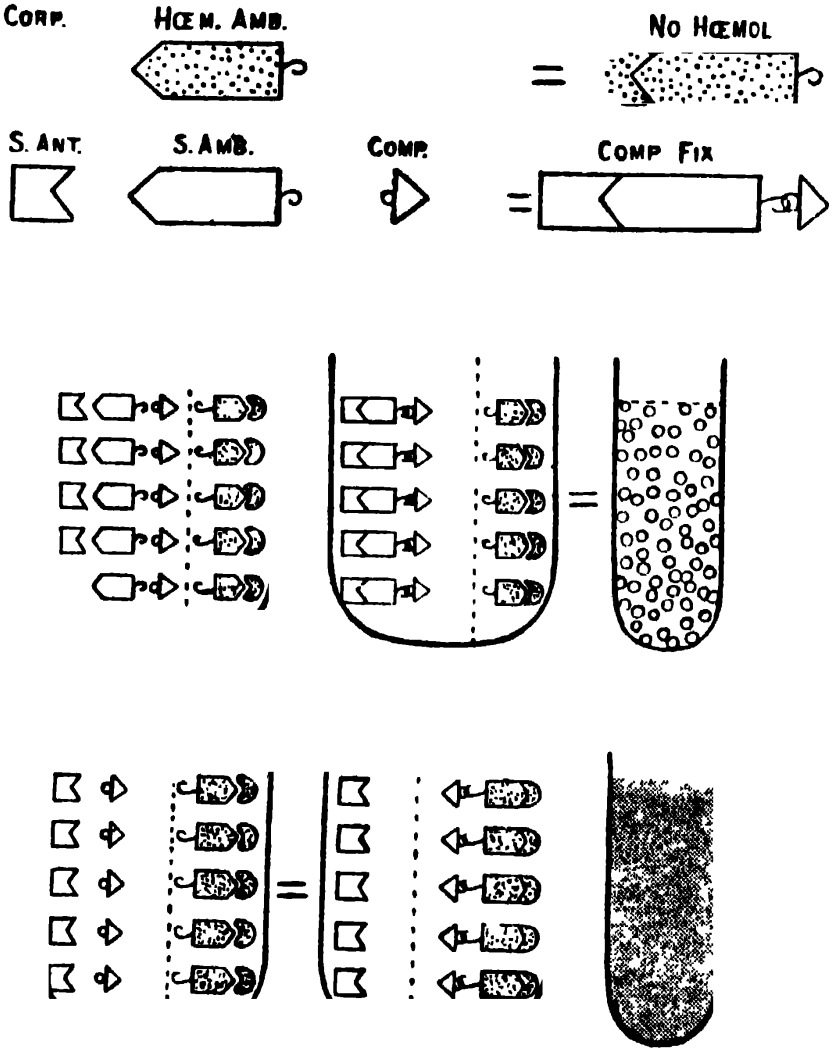


Fig. 147. Diagram illustrating the Wassermann Reaction.

Corp—Corpuscles (hæmolytic antigen). Hœm. Amb—Hæmolytic amboceptor. No Hœmol—No hæmolysis due to want of the complement = "sensitised corpuscles." S. Ant—Syphilitic antigen. S. Amb—Syphilitic amboceptor. Comp—Complement. Comp. Fix—Fixation of complement.

A—Represents the reaction with known *syphilitic* serum containing spyhilitic amboceptor.

S. Ant+S. Amb.+Comp.=Complement fixed+sensitised corpuscles=no hæmolysis.

B—Represents reaction with known *non-syphilitic* serum containing no syphilitic amboceptor.

S. Ant+Complement=no fixation of complement+sensitised corpuscles= hæmolysis.

tively easy. Yet of the number of precipitation tests proposed, none have received universal application. Kahn's test has been tried by a number of workers, who find generally a close parallelism between the results of this and Wassermann test. Kahn test is simpler than the original Sachs Georgi or the Sigma test and the extract contains more cholesterol.

Fresh ox-heart freed from fat, fibre and vessels, is minced and thoroughly dried with a fan. It is then extracted with ether to remove fat, the ether being renewed daily for three days and then filtered off. The residue is dried again until free from ether, and is then extracted with 5 times its amount of alcohol (96 per cent.), for nine days in the ice-chest and for one day at room temperature, before being filtered. To the filtrate is added cholesterol in the proportion of 0.4 per cent. Kahn has recently modified his test in certain important particulars in order to increase its delicacy and to overcome the necessity of incubation overnight. He claims that by his modified method, the reaction is complete in 95 per cent. of positive cases within 5-10 minutes and in the rest within an hour. Serum, antigen, and normal salt solutions must be so used as to assure as high degree of concentration as the mechanism of the test permits. The main effects of concentration appear to be (1) *Immediate* formation of precipitate; (2) The nature of the precipitate is influenced by the degree of concentration; (3) Next in importance to concentration appear to be the instability of A. S.—Antigen-Saline—mixture which is used with the serum. The antigen-saline mixture used in Kahn's test contains a precipitate but this precipitate is so unstable that it readily dissolves in the serum or salt solution. The strength of the antigen to be used in the test is the lowest dilution in which the precipitate can be dissolved with a little 0.85 per cent. salt solution. A series of dilution of antigen is prepared by adding to 0.5 c.c. antigen in each of a number of tubes containing 0.3 c.c., 0.4 c.c., 0.5 c.c. and 0.6 c.c. of saline. From each of the dilutions in which there is a *precipitate,* a sample of 1 c.c. is taken and added to 0.2 c.c. saline in a separate test tube. The lowest dilution of which the sample thus treated, goes into complete solution, is chosen for the test—usually it is mixture of equal parts of antigen and saline. The actual amounts of serum and antigen-saline mixture used in the test do not matter much but the *correst proportion of Serum* to *antigen-saline mixture* must be maintained. The three proportions of serum to *antigen-saline mixture* (3:1, 6:1, 12:1) are adopted in the routine test.

Routine test

The sera to be tested are inactivated and the tubes are set up and numbered before preparing the antigen dilution.

Preparation of the antigen dilution.—

1. Add 1cc. antigen to a standard antigen tube. (5.5 cm×1.5 cm.)
2. Add the amount of saline (0.85%) indicated by titration to a similar tube.
3. Pour the saline into the antigen tube as rapidly as possible and pour the mixture back and forth six times.
4. Allow this antigen dilution to stand for at least ten minutes before use. It should be used within half an hour after the dilution.
5. As a control of the proper mixing of the antigen and salt solution take 0.05 cc. of the antigen dilution into a tube and add 0.1 cc. of saline. After shaking vigourously for 15 seconds, the mixture should appear opalescent, the precipitate showing complete solution.

Performance of the test.—

A rack containing three parallel rows of holes is taken.

1. Measure 0.05, 0.025 and 0.0125 cc. respectively of antigen dilution into 3 tubes (7.5 cm.×1 cm.).
2. Add 0.15 cc. of clear inactivated serum into each tube. Mix the antigen dilution and the serum thoroughly.
3. Shake the tubes vigourously for 2 minutes and put in a water bath for 15 minutes.
4. Add 0.5 cc. of saline to each tube. The tubes are shaken for several seconds to permit thorough mixing and then allow to stand in room temperature for 5 minutes.
5. The result is taken in front of a window against a dark back-ground and with the tube held almost horizontally above the eye level.

Readings are recorded as ++++, +++, ++, + and — on the basis of the distinctness of the precipitate and clearness of the medium.

++++ represents a complete precipitation reaction, the precipitate being suspended in a quite clear medium.

+++ represents a definite precipitation but the medium is not quite clear.

++ represents a precipitation although definite but weak and is present in a cloudy medium.

+ represents weaker precipitate in a cloudy medium.

— represents no precipitate and the medium is quite opalescent.

Testing for Blood Group.

Before a blood transfusion is done it is necessary to make sure that the serum of the donor does not agglutinate nor hæmolyses the corpuscles of the recipient, or vice versa. Serious results may otherwise ensue. Since "hæmolysis never occurs when agglutination is absent," the selection of proper donor from the point of view of agglutination determines the other also.

Tests have shown that among human beings all individuals fall in four groups, having the following characteristics:—

Group I (5 per cent. of individuals) :—

Serum agglutinates cells of no other group.
Cells are agglutinated by sera of Groups II, III, IV.
As donors may be used for Group I only.
As recipients may receive from Groups I, II, III, IV.

Group II (40 per cent. of individuals) :—

Serum agglutinates cells of Groups I, III.
Cells agglutinated by sera of Groups III, IV.
As donors may be used for Groups I, II.
As recipients may receive from Groups II, IV.

Group III (10 per cent. of individuals) :—

Serum agglutinates cells of Groups I, II.
Cells agglutinated by sera of Groups II, IV.
As donors may be used for Groups I, III.
As recipients may receive from Groups III, IV.

Group IV (45 per cent. of individuals) :—

Serum agglutinates cells of Groups I, II, III.
Cells are agglutinated by no Group.
As donors may be used for Groups I, II, III, IV.
As recipients may receive from Group IV only.

It is, of course, preferable to have as donor and recipient members of the same group, but when it is impossible, members of another group may be chosen in accordance with the above. The agglutinative power of the donor's serum may be disregarded because it is so promptly diluted by the serum of the recipient.—(*Medical War Manual*—U.S. Army).

The blood should be tested every time the transfusion is given, even when, the same donor is used repeatedly for the same recipient. The four groups and their inter-relationship can be best explained in the following chart devised by Sanford.

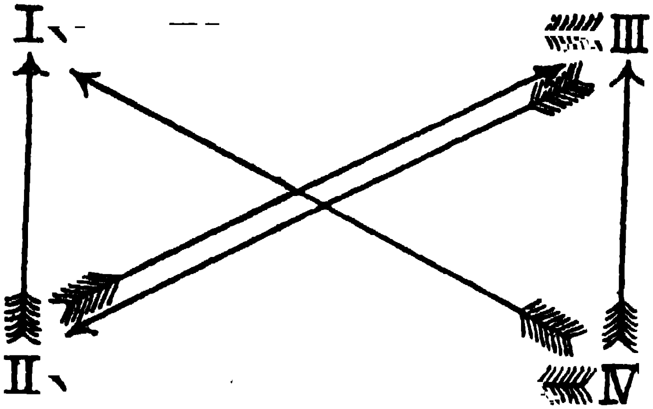


Fig. 148. Chart devised by Sanford.

The serum of any group will agglutinate the corpuscles of those groups towards which its arrows point. Thus the serum of an individual belonging to the group IV will agglutinate red blood cells of any other group, while serum of group I lacks the agglutinating power Several subgroups have been identified.

EXAMINATION OF FÆCES

Normal fæces is composed of—(*a*) Water. (*b*) Non-digestible and undigested particles of food, *e.g.*, vegetable cells and fibres, particles of meat, starch granules, etc. (*c*) Products of the digestive tract, *e.g.*, mucus, altered bile pigment, epithelial cells, etc. (*d*) Decomposition products, *e.g.*, indol, skatol, fatty acids, various gases, etc. (*e*) Bacteria (generally non-pathogenic). Pathologically—Blood, pathogenic bacteria, animal parasites and their eggs; concretions, etc.

Collection of material.

The sample must be examined very soon after it is voided especially in cases of examination for protozoa. It should be collected in a glass or metal vessel and sent to the laboratory for examination but it is always desirable to see the fæces in bulk.

Physical examination.

1. *Colour.*—It is normally dark brown and is due to stercobilin.

(*a*) Clay-coloured fæces are found when the pigment is absent in the fæces as in the case of obstruction of the bile ducts—obstructive jaundice. (*b*) Rice-watery stools—absence of bile and presence of flakes of mucous membrane as in a case of cholera. (*c*) Hæmorrhagic *e.g.*, in dysentery. (*d*) Tarry stools—when the hæmorrhage is high up and is partly digested—as in gastic or duodenal ulcer. (*e*) Green stools—generally in diarrhœa of infants. (*f*) Pea soup—in typhoid.

(*g*) Fatty or greesy appearance is due to presence of fat when absorption of fat is defective, *e.g.*, in pancreatic disease.

2. *Consistency.*—Normally semi-solid. When very hard it is known as scybala. Stricture of rectum makes it ribbon-like in shape.

3. *Odour of stools.*—Due to skatol and similar derivatives.

4. *Mucus.*—It is present in small quantity in cases of constipation and is of little importance. Cases of piles or other surgical diseases contain mucus tinged with blood or free blood. If present in large amount and intimately mixed with fæces it indicates irritation of small intestine, if in scattered masses it indicates irritation of large intestine.

5. *Pus.*—Indicates ulceration of large intestine especially rectum. When in considerable amount it indicates bursting of an abscess in intestines.

6. Presence of undigested food particles, worms, gallstones or other concretions are to be noted.

Chemical examination.

Stercobilin.—This is normally present in fæces and produces the normal colour. It is tested by the following method. Extract the fæces with chloroform, and concentrated HCl containing a little concentrated HNO_3. Examine with the spectroscope.

Bile acids.—Extract the fæces with alcohol, evaporate, dissolve the residue in NaOH. To 5 c.c. of this add 1 c.c. of 10 per cent. lævulose solution and allow concentrated H_2SO_4 to form a layer at the bottom of the tube. A ring of rose-pink colour indicates bile salts.

Occult blood.—(Blood pigment)—caution—meat must not be taken within 48 hours before the collection of the specimen of fæces.

(*a*) Weber's test.—The fæces (preferably fluid fæces) is mixed with 2 or 3 c.c. of glacial acetic acid and then shaken with sulphuric ether. The ether has a tokay-wine colour if blood be present.

(*b*) Benzidin test.—Emulsify fæces with water, add half its volume of glacial acetic acid, shake thoroughly, extract with ether by reversing the tube 20 or 30 times without causing froth. The blood has now been converted into hæmatin acetate. Pipette off the ethereal extract, add ½ c.c. glacial acetic acid, equal amount of saturated solution of benzidin in glacial acetic acid and equal part of hydrogen peroxide. A deep blue colour appears at the junction of the fluids if blood is present.

Reaction.—Is usually amphoteric or feebly alkaline when fresh It becomes acid due to fermentation after a few hours. In pancreatic diseases the reaction may be acid. In chronic enteritis or colitis the alkalinity is more marked. In baciliary dysentery the reaction is alkaline whereas in amœbic dysentery it is acid. It is strongly alkaline in cholera.

Fat.—A small quantity of both neutral fat and fatty acid crystals are present normally. Any excess is determined by Cammidge's method (for details of the method see special works).

The relation of unsaponified to saponified fat is normally about 10-15 per cent. In pancreatic disease the unsaponified fat is in excess. The saponified fat is in excess in biliary obstruction. In cancer of the pancreas with jaundice, the relations are nearly equal.

Microscopical examination.

1. *Undigested food particles*:—

(*a*) Large number of vegetable cells and fibres are present normally. (*b*) Large number of starch granules or undigested muscle fibres or elastic fibres indicate disease of small intestine or pancreas. (*c*) Fat—is present as globules or fatty acid crystals. An excess of fat indicates—(*i*) Disease of the pancreas. (*ii*) Deficient fat absorption due to intestinal disease. (*iii*) Deficiency of bile. Charcot-Leyden crystals are often found in amœbic dysentery and ankylostomiasis.

2. Other cells and crystals, blood corpuscles, epithelial cells, crystals of calcium oxalate and phosphates are often met with.

Bacteriological examination.

Emulsify the fæces in sterile saline or preferable in broth. Take a platinum-loopful of emulsion and spread on a MacConkey's plate and incubate for 24 hours. The spreading on the plate can also be done by a glass rod bent at right angles, the long arm is used to hold it and the short arm as the spreader. In order to get isolated colonies it is better to use 3 plates each one being inoculated with the same spreader one after the other with the same portion of fæces taken from the first plate; or four quadrants of a large plate can be used successively.

24 hours after inoculation, the colonies of B. coli will appear red, and those of other intestinal organisms, colourless. Take one of these colouress colonies, and inoculate into glucose and mannite tubes. If glucose is fermented with production of only acid and no gas, it is either B. typhosus, or, the dysentery bacillus. If both acid and gas produced, it is either paratyphoid, or Gærtner. Typhoid can be differentiated from dysentry organisms by their motility. Dysentery organisms are non-motile. Paratyphoid and Gærtner groups cannot be differentiated by other sugar reactions. Agglutination test with high titre serum is required for their differentiation. The sugar reaction is tabulated as follows:—

Fæces inoculated in MacConkey's medium.
(*Bile salt—neutral red—lactose medium*)

Acid—red colour—B. coli

Not acid-colourless colony
Glucose

Acid
B. typhosus (motile)
B. dysenteriæ (non-motile)
Mannite

Acid & Gas
Para A
Para B
Gærtner

Acid
B. flexner

Not acid
B. shiga

In case of Cholera:—

(*a*) Hanging drop preparation shows characteristic vibrios.

(*b*) Inoculate Dunham's peptone water. The vibrios will grow superficially, often, with the formation of a pellicle, other intestinal organisms will grow in the deeper layers. Add concentrated H_2SO_4 a few drops only—red colour, (*cholera red reaction*).

(*c*) Inoculate *Dieudonne's* media (Blood-alkali-agar) or taurocholate agar. No organism other than cholera vibrio will grow.

(*d*) Confirm the identification by agglutination reaction.

Tubercle bacillus.—Very difficult to detect in fæces. Make thick emulsion with antiformin, centrifugalise. Wash deposit with water several times. Examine the upper part of the deposit—(*a*) By staining with Ziehl-Neelsen method. (*b*) Cultivation. (*c*) Inoculation into guinea-pig.

Examination for Protozoa

Note:—1. Protozoa, especially the amœbæ, rapidly die outside the intestines. Hence always examine fresh samples. Amœbæ rarely survive more than 2 or 3 hours. 2. There should be no urine mixed up with fæces, as it kills the protozoa readily. 3. Free-living amœbæ and flagellates are often present in the fæces from the water used to wash the bed pan. 4. Always use freshly *boiled* saline to emulsify the fæces, as old saline may contain free-living protozoa.

5. Motile protozoa are found in liquid fæces whereas cysts are found in formed fæces. 6. Blastocystis—a form of vegetable organism often looks like cysts of protozoa.

Examination in fresh saline.

Take a portion of the fæces, especially the mucus or blood stained part, and, if thick, make an emulsion with a drop of boiled saline, put the cover slip on. See that the film is very thin (so that print can be easily read through it). Examine under high power. If the microscopical field is seen full of cellular elements, especially polymorphonuclears, it is probably a case of bacillary dysentery. In amœbic dysentery, cellular elements are scanty, and leucoytes are few. The red corpuscles are crenated and few.

Search for amœba.

Don't give any opinion unless the movement is distinctly seen (macrophages are often mistaken for amœba as they often ingest red blood corpuscles). When one amœba is seen in moving condition, look for red blood corpuscles in it; any entamœba with red blood corpuscle inside is E. histolytica. Then look for cysts, flagellates or Charcot-Leyden crystals. Make another thin emulsion with iodine solution (Iodine 1, KI—2, water 100 parts). The iodine preparation beautifully shows the cystic forms. For detailed study or for permanent specimens staining with Heidenhein's iron-hæmatoxylin method is the best.

Dobell's technique.

1. The film should be handled very gently as it is liable to be washed off easily. Spread a thin emulsion of fresh fæces on a slide with platinum loop. When the film is just on the point of drying, place it gently in—

Schaudinn's fixative (saturated solution of corrosive sublimate in distilled water) 2 parts. Absolute Alcohol 1 part. Mix and add glacial acetic acid 5 c.c. to every 100 c.c. of the above mixture. This fixative is best used at a temperature of 60°C. Keep the film in this fluid for 10-20 mins.

2. Take out the slide and remove most of the fixative by rinsing with 50 per cent. alcohol. Put in 70 per cent. alcohol in which a few drops of iodine solution have been added so as to tinge the alcohol port wine red. Keep the film until it is brown. The iodine changes the mercury to mercuric iodide.

3. Remove the mercuric iodide by soaking in rectified spirit or absolute alcohol for a day or two which will also harden the film and maceration or shrinkage during subsequent manipulations will be prevented.

4. Pass through graduated alcohols—70 per cent., 50 per cent., 30 per cent., and then distilled water.

5. Mordant the film in a watery solution of iron alum (2.5 to 4 per cent.) for not less than 6 hours. Rinse in distilled water.

6. Stain with "ripe" hæmatoxylin (*i.e.*, hæmatoxylin oxidised) for 6 hours or more, until it is over-stained, *i.e.*, the film becomes jet black.

"Ripe" hæmotoxylin is prepared by making a 0.5 per cent. solution of Grubler's hæmotoxylin and exposing it to sunlight for several weeks until the solution becomes good brown colour.

Differentiate by extracting the stain with the iron-alum solution (1 per cent. solution). This is the difficult and important part of the technique. Iron-alum removes the stain from the cytoplasm and not from the chromatin. The differentiation is done by occasionally rinsing the film with water and examining under microscope to watch the process. Vegetative forms decolourise early, cysts take a long time, even hours.

7. When the staining is satisfactory, wash first in distilled water, and then, in tap water for half an hour.

8. Counterstain with eosin, or any other plasma stain; wash, dehydrate gh ascending grades of alcohol 30 per cent., 50 per cent., 70 per cent., ed spirit, absolute alcohol, absolute alcohol and xylol and then xylol. Mount in neutral canada balsam with very thin cover glass.

CEREBRO-SPINAL FLUID

ion of material.

btained by lumbar-puncture during life; collect in a sterile test tube. About 5-6 ccm. of the fluid will be sufficient.

Physical examination.

Appearance.—Normally, quite clear and colourless. It may be lescent, turbid or purulent in acute meningitis. Red colour indis blood, due to, either injury to a blood-vessel during operation, or meningeal hæmorrhage.

Tension.—Normally, a flow of a drop per second through the lumbar-puncture needle. Pressure of flow 5 to 7.5 mm. of Hg. More than this, indicates increased intracranial tension due to meningitis, hæmorrhage, cerebral tumour, etc.

Specific Gravity.—About 1,006. Increased in meningitis.

Chemical examination.

Reaction is always alkaline. The following substances are found in normal or abnormal condition. (*a*) Glucose—45 to 65 per cent. of the blood-sugar is present in the c.s. fluid. This is lowered in meningits. (*b*) Urea—0.15 parts per 1,000—increased in uræmia. (*c*) Cholin—a trace is generally present. (*d*) Protein—both albumin, and a trace of globulin. In meningitis there are albumin, increased amount of globulin and nucleoproteins. Total protein of more than 50 mg. is pathological.

Test for Globulin.—(Noguchi's test) 0.2 c.c. of the cerebrospinal fluid is added to 1 c.c. of a 10 per cent. solution of butyric acid in normal saline and boiled for several minutes. 0.1 c.c. of normal caustic soda solution is then added and boiled again.

A flocculent ppt. indicates excess of globulin. Globulin is increased in cerebro-spinal syphilis and poliomyelitis.

Colloidal Gold Test.—(Lange's)—also indicates increase of protein constituents. All glass wares used must be chemically clean.

Technique.—Take 11 test tubes in a rack. Prepare 0.4 per cent. saline. Put 1.8 c.c. in the first tube and 1 c.c. in each of the other 10 tubes. Put 0.2 c c. cerebro-spinal fluid in the first tube and mix with the saline making a dilution of 1 in 10. Withdraw 1 c. c. from the first tube and add to tube 2, making a dilution of 1 in 20. Withdraw 1 c.c. from the second and add to the third. Repeat this up to tube No. 10 making a dilution from 1 in 10 to 1 in 5,120. Tube 11 should not contain any cerebro-spinal fluid and will serve as a control. Add 5 c.c. of the colloidal gold reagent to each of the 11 tubes. Allow to stand for 12 to 24 hours at room temperature and then note the colour reactions.

Tube 11 serves as control and the colour will be orange red and perfectly transparent. Note change of colour for blue or lilac. If the colour changes are present in the first five tubes (dil. 1 in 10 to 1 in 160) the term "Paretic curve" is applied. If the colour change is present in the tubes from 3 to 6 (1 in 40 to 1 in 320) it is called the "Luetic curve." If in tube from 6 to 10 (1 in 320 to 1 in 2,560) it is called the "Meningitic curve"

A positive "paretic curve" indicates that the patient is suffering from general paralysis of insane. Luetic curve indicates cerebro-spinal syphilis. Meningitic curve indicates meningitis.

Cytological examination.

Centrifugalise the fluid, examine sediment on slide as a film preparation. The fluid is normally free from any cell except the few red cells and leucocytes introduced through the needle from injury of the vessels during puncture.

A large number of polymorphonuclear cells indicate bacterial infection (except tuberculosis and syphilis), *e.g.*, strepto, pneumo, meningo, staphylococci.

A large number of lymphocytes indicate tuberculous or syphilitic affection of the meninges or protozoal diseases, *e.g.*, trypanosomiasis.

Presence of atypical cells indicates malignant growth.

Total Count of Cells.—Take a portion of the cerebro-spinal fluid and tinge with the smallest quantity of methylene blue or gentian violet and count the cells with the hæmocytometer. In samples containing blood, both red cells and leucocytes are to be counted and subtract one leucocyte for every 650 red blood corpusles.

Interpretation. Normal cell count is 5 cells per c.mm. *Pleocytosis* is increased cell count of cerebro-spinal fluid the cells being either polymorphonuclears or lymphocytes.

High polymorphonuclear cell count indicates bacterial infection of the meninges or nervous system. High lymphocyte count with clinical signs of meningitis generally indicates tuberculous or influenzal meningitis or poliomyetitis. In case of cerebro-spinal syphilis and tuberculosis the following are the cell count.

Tabes ..	10-30 per c.m.m.	Tuberculosis ..	below 100
G. P. I. ..	30-60 per c.m.m.	C. S. S. ..	more than 100

Bacteriological examination.

Collect the fluid in a sterile container, centrifugalise, take the sediment, and examine as a hanging drop preparation; stain films with Meth. blue, Gram and Leishman. Culture and inoculate laboratory animals. The following organisms may be present:—

Pneumococcus, Streptococcus, Meningococcus, Tubercle Bacillus, Staphylococcus, Pneumococcus, B, typhosus, B. influenza, Spirochæta pallida, and the trypanosomes.

Serological.

The fluid should be examined fresh. No heating is required as it does not contain any hæmolytic complement.

For details see Blood—Wassermann Reaction

BACTERIOLOGICAL EXAMINATION OF THROAT

Collection—Collect by means of a sterile swab from the throat—preferably, from any white patch if present.

Organisms expected to be present.

Staphylococcus, strepto, pneumo, diphtheria, pseudo-diphtheria, meningococcus, influenza B. yeast, spirochæta vincenti, fusiform B., sarcinæ, monilia, organisms of thrush (O. albicans).

N.B.—Examine especially for B. diphtheria and use Neisser's stain.

For Examination—See under general methods of Bacteriological work.

Interpretation.—A report " diphtheria bacilli present " from the smear taken from an acute inflammatory condition of the throat must be taken as diphtheria. A negative report means that no morphologically recognisable diphtheria bacilli can be seen and so this should be taken as no evidence. If the symptoms are like diphtheria it must be treated as diphtheria and a second examination should be advised.

In a convalescent diphtheria a single negative finding does not mean that the patient is free from infection. It must show three consecutive negative results. A negative report must be controlled by Schick test. A positive Schick test with negative swab indicates that the case is not diphtheria while a negative Schick test with a negative swab indicates that the case is probably one of diphtheria. A nasal swab may also be examined as control.

EXAMINATION OF SPUTUM

Familiarize yourself with the structures (buccal epithelium, leucocytes, food particles, the so-called "salivary corpuscles," saprophytic bacteria, spirochætes, etc.) present in the normal mouth and appear in sputum as contamination. Spread uniformly between two slides. Fix by heat and stain by Ziehl-Neelsen method.

Organisms expected.

Tubercle bacillus, staphylo, strepto, diplo, pneumococcus, M. cattarrh, M. tetragena, influenza B. B. pertussis, B. pestis, B. anthracis, Freidlander's B. actinomyces, streptothrix, moulds, spirochætes, etc.

Collection.

Either morning sputum or whole amount for 24 hours to be examined. Collect in a clean wide-mouthed phial without any disinfectant.

Examination.

Spread the sputum on a Petri dish in order to get it in thin layer. Select with the naked eye or with a hand lens a small, thick, cheesy particle from the sputum and not the mucus only.

A black back ground gives more convenience.

Physical examination.

Quantity.—Varies a good deal. There may be none or it may be very large (up to 1,000 c.c.) in bronchiectasis or tuberculous cavities or rupture of abscess or empyema.

Colour.—Ordinarily colourless and translucent but may be 'rusty' or red (due to the presence of blood), or whitish, yellowish or purulent.

Consistency.—Usually mucoid but may be serous, purulent, seropurulent, or mucopurulent. In bronchiectasis the sputum shows a peculiar tendency to form 3 sharply defined layers when allowed to stand in a tall vessel.

Smell.—A foul-smelling sputum is very suggestive of bronchiectasis specially when large number of organisms of different kinds are present in it.

Microscopical examination.

Elastic fibres, Charcot-Leyden crystals, Curschmann's spirals, ray-fungus of actinomycosis and pus cells are often better identified when examined unstained. Red blood cells, leucocytes, lymphocytes, eosinophiles, epithelial cells are also found.

Antiformin method.

Used only when tubercle bacilli are few in number. Add one part of antiformin (liq. sodi chlorinatæ and NaOH) to 4 parts of sputum. Keep in incubator for two hours. NaOH dissolves the mucus, liq. sodi. chlorinatæ kills the other organisms except T. B. Centrifugalise, wash the sediment with sterile water 2 or 3 times, divide into three parts. 1. Make smears and stain. 2. Culture for T. B. on glycerine, egg or blood serum media. 3. Inoculate a guinea-pig.

Other microscopical findings.

1. Fragments of pulmonary tissue, elastic fibres. They are best detected after boiling sputum with caustic potash which dissolves the mucus leaving the elastic fibres unaffected. They appear as wavy, refractile fibres with uniform thickness often arranged as if surrounding an alveolus. 2. Curshmann's spiral—found in asthma. 3. Charcot-Leyden crystals. 4. Other parasites—*e.g.*, lung fluke.

Albumen Test For T. B.—Take 10 c.c. of fresh sputum add equal quantity of water and 2 c.c. of 3 per cent. acetic acid solution, Filter. Test the filtrate for albumen. Presence of albumen indicates tuberculosis. Active tuberculosis shows 0.2 per cent. albumen or even more.

Interpretation.—A positive finding of tubercle bacilli in sputum is fairly easy by the Ziehl Neelsen method when they are present in fair number. But if they are scanty antiformin method will reveal them. In doubtful cases of tuberculosis with negative tubercle bacilli finding, fresh samples of sputum should be examined daily for a week.

The number of organisms present in a sample gives no idea regarding the acuteness of the infection. A chronic case with apparent good health may show large number of organisms in the sputum. Secondary organisms are generally found in late stages of pulmonary tuberculosis.

The interpretation on the findings of organisms other than tubercle bacilli is difficult and depends to a great extent on the efficiency with which contamination from the mouth has been prevented. Presence of unusual substances *e.g.*, Charcot—Leyden crystals, Curschmann's spirals etc., indicate asthma.

BACTERIOLOGICAL EXAMINATION OF URINE

Collection of material.—Catheter specimen preferable but essential for females.

Organisms expected.—Staphylo, strepto, B. coli, B. typhosus, gonococcus, paratyphosus, A. and B., Proteus vulgaris, M. melitensis, T. B., smegma B., spirochætes, diphtheroids, yeast, moulds, etc., Bilharzia eggs, T. vaginalis, Echinococcus.

Inoculate.—Ordinary agar tubes, MacConkey plate in case of Typho-coli group of organisms.

EXAMINATION OF PUS

Collection.—Collect by means of a platinum loop or sterile swab or hypodermic syringe.

Organisms expected.—Staphylo, strepto, pneumo, gono, M. catarrh, diphtheroids, anaerobic organisms.

Make smear on slide, stain with methylene blue and by Gram's method; culture on agar.

EXAMINATION OF SMEAR FOR SPIROCHÆTES

Fontana's method for demonstrating spirochætes.

Solutions required.—(1) Fixative:—Acetic acid 1 c.c. Formalin 2 c.c. Dist. water 100 c.c. (2) Mordant:—Carbolic acid 1 grm. Tannic acid 5 grams. Dist. water 100 c.c. (3) Ammoniacal Silver Nitrate:—Make 1½ per cent. solution of $AgNO_3$ in distilled water. Add 10 per cent. ammonia until the precipitate formed just dissolves. Add more silver nitrate solution drop by drop until the precipitate returns and does not re-dissolve.

Method.—(1) Treat the film 3 times, 30 seconds each time, with the fixative. (2) Wash off the fixative with absolute alcohol and allow alcohol to act for three minutes. (3) Drain off excess of alcohol, put it over the flame to burn the remainder. (4) Pour mordant, heat to steaming and then allow to act for ½ minute. (5) Wash well in distilled water. Dry. (6) Treat with ammoniacal silver nitrate, heat to steaming for ½ minute, till the film becomes brown. (7) Wash well, dry, mount in balsam with coverglass on, as some immersion-oils, cause the film to fade at once.

The spirochætes are stained brownish-black on a yellowish background.

CHAPTER XXII

GENERAL METHODS FOR BACTERIOLOGICAL WORK

Routine procedure.

1. *Preparation of a wet film.*—Put a small drop of water on the slide and a minute portion of the material to be examined with a platinum loop. Put a clean cover glass on and examine under the high power (⅙ in. or 4 mm. objective). Focus a small air bubble and then try to focus the organisms.

2. *Preparation of a dry film.*—Spread the minute portion of the material on the slide with a tiny drop of water, allow it to dry in air, and fix by passing, with the film upwards, three times through the flame of the gas burner or a spirit lamp.

Methods of Staining.

Ziehl-Neelsen method of staining.

Prepare a film, dry in air, fix by passing film over the flame. Hold the slide film upwards, and carefully drop carbol fuchsin sol. upon the film so as to cover it. Hold the slide over flame at such a distance that the stain just steams gently; continue heating for 5 minutes, carefully avoiding boiling. Then wash well under a tap and place in 5 per cent. sulphuric acid. Wash well in water. The film should be of faint pink colour. The process removes the colour from all bacteria except the acid-fast organisms, *e.g.*, tubercle bacillus, lepra bacillus. Finally stain for 30 seconds with an aqueous solution of methylene blue. Wash in water and allow it to dry in air. Examine under an oil immersion lens.

Gram staining.

Dry and fix a film and then stain with carbol methyl violet for 5 minutes: wash; pour Gram's iodine (iodine—1, pot iod.—2, water 300) keep for 1 or 2 minutes, decolourise with absolute alcohol; wash, counterstain with 1 per cent. eosin, very weak carbolfuchsin, or Bismarch brown.

Procedure. 1. Make a wet film and examine for motility and general character. 2. Stain with methylene blue or carbol fuchsin and note the form, arrangement, size, capsules, spores, etc. 3. Stain with Gram's method and note whether it is Gram-positive or not. 4. Inoculate with a sterile platinum loop one agar culture tube with the material to be examined. Incubate for 24 hours, and note the cultural peculiarities.

1. **Examination of unstained wet film.** (*a*) Shape—Cocci—dot shaped. Bacilli—rod shaped. Spirila—cork-screw shaped. (*b*) Size—Cocci—all measure about 1 μ except Micrococcus melitensis—0.4 μ. Bacilli—all measure 2-5 μ $\times$0.5 μ except B. anthracis which is 4—10 μ. (c) Motility—B. tetani, B. pyocyaneus and all intestinal bacilli except dysentery organisms (shiga, flexner, etc.) are motile. Rest—Non-motile.

2. **Stained specimen.** (*a*) Meth. blue.—All take stain. Note shape and size as above. Capsule-present in Pneumococci and Pneumo-bacilli, B. *Æ*rogenes capsulatus. (*b*) Gram-positive cocci are staphylo, strepto, pneumo, tetragena. Gram positive bacilli are the acid fast, spore forming, diphtheria and diphtheroids.—Rest—Gram-negative. (*c*) Special stain—Ziehl-Neelsen method for acid fast organisms and spores, Neisser's stain for B. diphtheria to differentiate from diphtheroids.

3. **Culture.** Agar—All grow well except gono, meningo, acid-fast, hæmophilic organisms and spirochætæ. Special media—*Media containing blood*—for gono, meningo, hæmophilic organisms, diphtheria, etc. *Media containing glycerine*—for T. B. *Egg media*—diphtheria, acid fast organisms. *Ghee or fat media or salt agar*—plague. *Ascitic fluid or animal tissue*—gono, meningo, spirochætæ. *Media containing sugars and indicators*—Typho-coli group. *Dieudonne's media; taurocholate agar media*—Cholera. *Dunham's peptone water*—Cholera.

4. **Inoculation to animals.** Rabbits and guinea-pig are commonly used. Mice for pneumococcus.

5. **Agglutination test.** Use specific high titre serum.

Isolation of Bacteria

1. Plating Method.

By diluting the material and inoculating it on agar plates. The material (pus, fæces, throat membrance, etc.), is distributed in a tube of sterile saline or broth with a swab or sterile camel hair brush or platinum loop. A little of the diluted material is taken up and several strokes are made on a petri dish containing agar. In case a swab is used, it should be expressed on the side of the test tube containing the material to get rid of the excess of the fluid. When plates are not available three agar tubes may be infected successively with the same swab, brush or loop, but charging them once only with the infected material. After 24 hours incubation the bacteria can be isoluted.

2. By using selective media.

By adding suitable chemicals and indicators to a solid medium and recognising and isolating the organisms by their reactions on the medium. Thus lead acetate is coloured brown with organisms producing H_2S, tellurite is reduced by B. diphtheriæ, litmus and neutral red changed by acid producing organisms.

Hæmolysis of blood may also be used as indictor. Sometimes inhibiting substances are added to the medium to prevent undesirable bacteria from growing. In neutral red--bile salt agar, we have lactose, neutral red and sodium taurocholate. Some bacteria ferment the lactose present in this medium with production of acid, others do not; this is indicated by the neutral red, which gives a red tint to the acid forming colonies. The sodium taurocholate acts as an inhibitor. This method is used extensively for isolating typhoid and dysentery bacilli from fæces.

In the same way antiformin is used to isolate B. tuberculosis inhibiting or destroying the other contaminating organisms. Similarly gentian violet is used to inhibit Gram positive organisms.

Investigation of a case for Coli-typhoid-dysentery bacilli.—

Plate stool or mucus from suspected stool on MacConkey (lactose neutral red bile salt agar medium). Incubate. The result is.—

1. Lactose fermenting colony (red) = coli group. All members of the coli group ferment both lactose and glucose with gas formation.

Non-lactose fermenting colony (white) = may be of dysentery group or of para-typhoid and enteritidis groups or B. fecalis alkaligenes, B. proteus vulgaris, B. pyocyaneus. All bacilli of these groups are Gram-negative. All of these bacilli are motile, except those of the dysentery group. Two of them, B. proteus and B. pyocyaneus form creeping colonies and can be differentiated as follows:—(*a*) B. proteus (ferments glucose), (*b*) B. pyocyaneus (produces pigment and does not ferment glucose). They also liquefy gelatine.

2. Non-lactose fermenters fall under four heads.—

(*a*) B. typhosus—Motile, forms acid in glucose and mannite. Confirm by agglutination test. (*b*) B. paratyphosus A. and B.—Motile, form acid and gas in glucose and mannite. Confirm by agglutination test. (*c*) B. dysenteræ and paradysenteræ. Non-motile. the former forms acid in glucose, but mannite is unchanged. The paradysenteræ group ferment mannite (For further differentiation of this group other sugar tests are necessary). Confirm by agglutination test. (*d*) B. facalis alkaligenes does not ferment any sugar and is highly ærobic.

3. Pathogenicity method.

By inoculating susceptible animals, *e.g.,* isolation of pneumococcus from sputum by inoculating mice and rabbits.

Method of Isolating Pneumococci from Sputum.

Take a small piece of mucus containing pneumococci—wash in saline and inject it into the peritoneal cavity of a mouse. When the animal is sick, a little of the peritoneal exudate is taken and stained to find if the growth of pneumococci is abundant—if not, further time is allowed till the pneumococci are abundant. The peritoneal exudate of the dead or killed mouse is plated on blood agar. Cultures and smears are also made from the heart's blood of the dead animal. Pneumococci, when put in the peritoneum of a mouse, grow more quickly than do other micro-organisms.

4. In case of **spore-forming organisms,** the material is heated to boiling or keeping it at a temperature of 80°C for 10 minutes so as to kill all non-sporing bacteria as also the vegetative forms of sporing ones. The spores will remain living which will grow when kept in a suitable media.

5. Filtration method. Filterpassing organisms may be isolated from ordinary bacteria by filtering the material through Pasteur-Chamberland or Barkefeld filters.

LABORATORY DIAGNOSIS OF INFECTIOUS DISEASES

Fungus diseases of the skin and hair.

Take scrapings, scales or hair on a slide, put a drop of 10 per cent. caustic soda or potash on; put a coverglass on and crush it between the slide and the coverglass; heat over the flame and examine. It is preferable to remove the fat by soaking the material with ether, then with chloroform, and, finally with water before putting in the caustic potash.

Actinomycosis and mycetoma.

Collect the grains, press between a slide and a coverglass and examine under the microscope.

Pneumonia.

1. Examine blood and sputum for pneumococcus and other organisms. 2. Determine the type of pneumococcus, if present. 3. Examine blood for leucocytosis.

Cerebro-spinal meningitis.

1. Cytological and bacteriological examination of cerebro-spinal fluid. 2. Culture blood for meningococcus. 3. Examine blood for leucocytosis. 4. Exclude cerebral malaria. 5. Examine swab from the naso-pharynx in suspected carriers.

Gonorrhœa. 1. Examine smear from urethral or conjunctival discharge for gonococcus inside pus cells. 2. Culture.

Tuberculosis.

1. Examine sputum, fæces and urine in cases of pulmonary, alimentary and genito-urinary tuberculosis respectively. 2. If organisms scanty examine by antiformin method. 3. Albumin test of sputum in pulmonary tuberculosis. 4. Inoscopy and histological examination in cases of tissues. 5. Estimation of opsonic index. 6. Tuberculin reactions:—

(*a*) Cutaneous reaction of Von Pirquet—scarify three areas on the forearm, rub on one old tuberculin(human), on the second bovine tuberculin and on the third 0.5 per cent. carbolic saline.

(*b*) Ophthalmic reaction of Calmette.

(*c*) Dermal reaction of Moro.

(*d*) Subcutaneous inoculation of 1|5 mg. of old tuberculin. In all forms watch for local, focal and general reactions.

7. Precipitin reaction. 8. Agglutination reaction. 9. Complement fixation test with standard emulsion of tubercle bacillus as the antigen.

Leprosy.

1. Microscopical examination of smears or section from the leprous tissue or nasal mucosa. 2. Remember Wassermann and tuberculin reactions may be positive. 3. Complement fixation reaction with antigen made from emulsion from leprous tissue containing a large number of the organisms or from culture, if available. Lepers may also suffer from tuberculosis and syphilis.

Anthrax.

1. In case of animals, examine spleen only. Full post mortem examination not advisable. Always examine as early as possible after death, otherwise saprophytic bacteria resembling anthrax will contaminate. 2. In man—examine smear from the lesion for the B. anthracis both in a stained film as well as in culture. 3. In shaving brush, wool etc.—Put material in 100 c.c. boiling water. Inoculate melted agar with small quantity of the water. Incubate

CHAPTER XXIII
IMMUNITY

Immunity is a condition which renders the body resistant to the invasion of infective process.

Immunity may be natural or acquired, active or passive.

NATURAL IMMUNITY

How is it that inspite of the presence of large numbers of pathogenic organisms on the surface of our body as well as in the internal cavities there is no infection? Although it is often very difficult to explain yet it seems to be due to one or more of the many facts. Some of them are as follows:—

(*a*) There are some infective diseases which particularly affect lower animals and never human beings even if experimentally inoculated and *vice versa*. Swine fever, rinderpest, etc., may be mentioned as examples. In some instances this is often relative as the man or the animal although so-called naturally immune can be infected with a fairly large dose. There are some diseases which are mainly confined to lower animals, *e.g.*, glanders, actinomycosis, etc., but will not spare man if he is infected with the respective organisms. (*Species immunity*).

Racial immunity.—The Jews have a great resistance to tuberculosis than other races.

Individual immunity—Different individuals present different amount of resistance to infective processes. Cholera is an example. When the infected water is taken by many people of the same household or even of the entire village, only certain persons get the disease.

Thus all grades of immunity exist in man or animals against various forms of infective processes.

(*b*) Some infective processes, *e.g.*, cholera, typhoid, etc., infect the human beings through the alimentary tract; gonococcus infects through the genito-urinary tract. Thus there are some selective paths, or channels through which the organisms must enter, and the individuals may be called immune to the infection if the organisms approach through points other than these susceptible ones.

(*c*) The intact unabraided epithelial lining of the skin, cavities and ducts offer a great resistance to the invading organisms, while microscopical abrasions on such surfaces may lead to serious results, *e.g.*, tetanus.

(*d*) In the stomach, on account of the presence of hydrochloric acid, active multiplication of the organisms is impossible, and in some instances, *e.g.*, cholera, its presence is fatal to the organism. Often this obstacle to infection becomes insufficient, owing to active motility of the stomach allowing the organism to pass to the intestine hurriedly, or, the presence of copious food, water or mucus protecting the organism from the action of the acid. In some instances, the acid may be deficient, as in fasting, or, in diseases of stomach, thus offering no resistance to the invaders.

(*e*) Some organisms, *e.g.*, tetanus, although infect through the skin, will not infect so long as there is no necrosis of the part or not until associated with other pyogenic organisms.

(*f*) The amount of the infective material also plays a great part as regards successful infection. Small doses in some instances will not infect but large doses may.

This is illustrated in cases of cholera, as large number of them are destroyed spontaneously.

The natural immunity may be overcome by any of the following factors:—

(*a*) Any devitalising condition lessening the resisting power of the individual, either of the whole body, or the local part. (*b*) Previous injury will lessen the immunity.

ACQUIRED IMMUNITY

This form of immunity may be either acquired by disease, or, artificially induced.

1. *Acquired by Disease.*—There are some diseases, *e.g.*, small-pox, typhoid, etc., one attack of which leaves the individual immune to a second attack for the rest of life. Some other diseases though they do not confer immunity for the whole life do so for a period varying from a few days to several years. There are others again, *e.g.*, pneumonia and rheumatic fever which instead of conferring any immunity, predispose to a second attack.

2. *Immunity acquired artificially.*—This may be either active, or passive. Active immunity is that form of immunity which is produced through the activity of the animal itself. Passive immunity, on the other hand, is the result of artificial transferrence of the ready-made immune bodies produced by the activity of another animal into the passively immune one.

ACTIVE OR DIRECT IMMUNITY

This is produced by the following methods:—

A. Inoculating Attenuated Living Organisms

(*a*) *By the passage through lower animals, e.g., vaccination against small-pox.*—The virulent small-pox virus when passed through cattle becomes very much attenuated. It must be remembered, that with other organisms, passage through lower animals usually leads to increase in the virulence, *exaltation* except in small-pox, in which case, *attenuation* takes place.

Formerly, vaccination was used from a man suffering from small-pox direct to the healthy. This was often followed by severe reaction, often, attack of small-pox and death. This process has now-a-days been replaced by vaccination obtained, from calf, which has been inoculated with the material derived from human being. This method has also removed the danger of transmission of other diseases from man to man through the lymph.

(*b*) *By allowing the organisms to grow in abnormal temperature.*—This is generally used in case of inoculation against anthrax as introduced by Pasteur. The organisms are allowed to grow at a temperature of 42°C. in broth for a period of about a month. This process attenuated the virulence of the virus. Inoculation of this attenuated virus leads to severe reaction in lower animals, but, at the same time, render them immune against more virulent doses. The immunity becomes so great, that the most virulent cultures can be injected without producing any ill effects. Thus, an animal, immunised in this way, will have great resistance against the organism which might have access to it by ordinary ways. This is a form of active immunity produced artificially. Pasteur is the father of this method, and, by it, he once saved France from great loss from destruction of sheep from anthrax.

(*c*) *By passing a current of air over the culture media.*—Haffkin's cholera inoculation. Haffkin used two cultures one weak and the other strong. The weak one was the attenuated virus obtained by passing a current of air through the culture medium. The weak virus is injected first leading to slight fever and local reaction. The stronger dose is then used after a few days.

(*d*) *Attenuated by drying.*—This is generally used in case of inoculation of rabies (Pasteur's method). The discovery of this form of immunity by Pasteur is a boon to the scientific world, more especially, because of the unknown nature of the virus. The organism of hydrophobia is still not definitely known. Pasteur found that whatever might be the nature of the virus, it is present in the central nervous system of the infected animal. His next difficulty was the long incubation period of the disease—about 6 months. In order to reduce this period, so as to put inoculation on a practical basis, he standardised the virulence of the virus (fixed virus) by inoculating series of guinea-pigs and rabbits subdurally by injecting through the trephine wound under the dura mater. After a few successive inoculations, the virulence was greatly exalted with a short and fixed incubation period. This, he used as standard for fixed virus. Attenuation of this living virus was done by drying the spinal cords of the animals in jars for varying periods. It was found that, the more was the drying of the cord, the more was the attenuation of the virulence of the virus. The dried cords were emulsified which were then ready for inoculation. He found that a cord dried for 12-14 days, when used for inoculation, will not produce any disease. After a few days, a strong dose, *i.e.*, from a cord dried for 10 days, will be easily tolerated, and, this, can be worked up until the strongest dose from a one-day cord will not produce the disease. By this means, he has been able to produce immunity against this virulent unknown organism. The virulence depends upon the period of drying as well as the amount inoculated. Pasteur's original method has now been improved by using virulent fresh cords only in minute quantities as the initial dose. The quantity is gradually increased according to the immunity produced.

(*e*) *Attenuated by keeping.*—Inoculation for plague. B. pestis is allowed to grow in broth for several weeks. This leads to production of involution forms, suspension of growth, and, utimately, distintegration of the organisms, liberating toxic products in the culture medium. The organisms are then subjected to a temperature of 70°C. for a few hours. This is then used in three different doses. The inoculation produces local reaction with fever occasionally to a marked extent.

(*f*) *Attenuated by allowing organism to grow in presence of some antiseptic.*—Chamberland and Roux attenuated the B. anthracis by allowing the organisms to grow in a medium containing a trace of carbolic acid (1 of carbolic acid in 600 of the medium).

(*g*) *Inoculating virulent organisms in a sub-lethal dose.*— The method is generally employed after the animal is partially immunised by inoculation with dead or attenuated organisms.

B. By Inoculating Dead Organisms—Vaccines.

(*a*) *Typhoid inoculation.*—The organisms are allowed to grow on agar from which emulsion is made in saline. The organisms are killed by keeping them at a temperature of 56°C. for one hour. The emulsion of the killed organisms is then kept in sealed jars with some antiseptics, *e.g.*, ½ per cent. carbolic acid as preservative. It is essential to note that all the organisms are killed. This is tested by inoculating a few culture tubes with this emulsion. The emulsion is then standardised by counting the number of organism present per c.c. of the emulsion.

Wright standardised the emulsion by regulating his dose to ⅓rd of the amount which would kill a guinea-pig weighing 250 grams, *i.e.*, ⅓rd the minimum lethal dose. He used this as the first dose to be followed after 10 to 14 days by a second one which is larger than the first one. Two doses are quite sufficient to produce immunity.

(*b*) *By sensitised vaccine.*—This is almost the same thing as the preceding one, except, that the bacterial emulsion has been kept in contact with the corresponding anti-serum. The anti-serum is subsequently removed completely by centrifuging repeatedly with normal saline. After such a treatment, the bacteria are sensitised,—the vaccine is termed *sensitised vaccine.* The advantage of sensitised vaccine over ordinary vaccine is, that in the former, the immunity develops more rapidly, lasts for a longer period, and local toxic effect after injection is greatly diminished.

C. Immunity Produced by Injecting Products of Bacteria.

(*a*) *Bacterial Toxins.*—This is probable in cases where the organisms liberate extra-cellular toxin, *e.g.*, in diphtheria and tetanus. In diphtheria and tetanus the toxic products can be separated from the body of the organisms by filtering them through Pasteur-Chamberland filter. The filtrate is free from organism and is highly toxic. An animal, usually the horse, is selected and a very small dose is injected. This is often followed by toxic symptoms which pass off readily. The animal is inoculated every week with stronger and bigger doses according to the degree of immunity produced in the animal. After a time the horse will be able to stand a dose many times the lethal dose without any inconvenience. Anti-diphtheritic and anti-tetanic sera are produced in this way.

(*b*) *Injection of Toxoids.*—Toxoids are substances which are not toxic by themselves but produce immunity when injected into an animal.

D. Immunity by Feeding.

Some authorities obtained active immunity in animals by feeding them with either the toxins or the vaccines *e.g.*, bilivaccines.

PASSIVE OR INDIRECT IMMUNITY

A. By Injection of Antitoxic Serum.

This is effected by injecting the ready-made anti-serum into the animal. It cannot be obtained by injecting the blood of naturally immune animals. As for example, most lower animals are immune to syphilis and gonorrhœa, but, their sera neither protect nor have any curative action to man. Active immunity takes a fairly long time to develop, *i.e.*, after several injections, whereas, passive immunity is produced almost simultaneously with the injection of antiserum; on the other hand, active immunity lasts longer than passive immunity. Passive immunity is generally produced in those cases after injection of antitoxic serum where the bacteria liberate an extra-cellular toxin as in cases of diphtheria and tetanus.

Diphtheria.—In cases of diphtheria, injection of the anti-diphtheritic serum containing the ready-made anti-toxin at once neutralises the toxin circulating in the body. It has also a great bactericidal power, by means of which, it destroys the organisms present in the throat. Thus, after the injection, the membrane ceases to spread, becomes separated within a short time, the organisms die out, and toxæmia disappears. Thus, this may be used both for prophylactic, as well as, for curative purposes. This sort of immunity lasts only for a short time, say, three weeks, and is passive in nature.

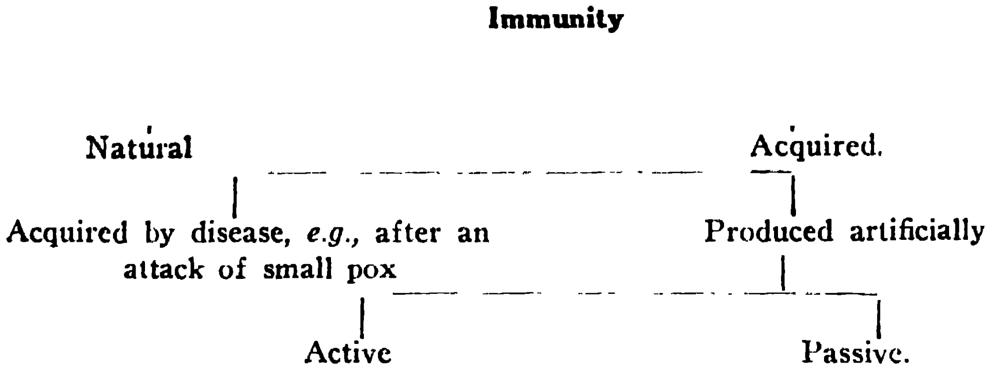
Tetanus.—This is more or less similar to what is observed in case of diphtheria, with the difference, that the anti-serum has got no power to kill the organism nor to neutralise the toxin when once it has become fixed with the cells of the central nervous system.

The injection of anti-tetanic serum only neutralises the toxin which is still free but cannot displace or neutralise the toxin already in combination with the cells.

B. By Injection of Anti-bacterial Serum.

This is illustrated in cases of anti-dysenteric and anti-streptococcic sera.

THE DIFFERENT FORMS OF IMMUNITY ARE SUMMARISED AS FOLLOWS



(*a*) By injection of antitoxic serum.
(*b*) By injection of antibacterial serum.

A. By living organism:—

(*a*) *Attenuated by passage, e.g.,* small-pox vaccination of Behring. (*b*) *Attenuated by growing in abnormal temp., e.g.,* Pasteur's anthrax inoculation. (*c*) *Attenuated by passing a current of air over culture*—Haffkin's cholera inoculation. (*d*) *Attenuated by drying, e.g.,* Pasteur's treatment of rabies (*hydrophobia*). (*e*) *Attenuated by keeping, e.g,* plague inoculation. (*f*) *Attenuated by allowing the organism to grow in presence of some antiseptic, e.g.,* Chamberland and Roux's anthrax inoculation. (*g*) Attenuated by inoculating in sublethal dose. (*h*) *With infective agents of full virulence, e.g,* variolation or inoculation with living small-pox virus from one human being to another.

B. By dead organism:—

(*a*) Vaccines, *e.g.,* typhoid inoculation. (*b*) Sensitised vaccines, *e.g.,* dysentery inoculation (Shiga).

C. By products of bacteria:—

(*a*) By injection of toxin of organism. (*b*) By injection of toxoids.

D. By feeding:—

By feeding the dead bacterial culture or their products. It is useful only for prophylactic purposes and helps very little as a curative agent.

THE DEFENSIVE MECHANISM OF THE BODY

This is managed by two factors—

(*a*) The bactericidal action of the blood. (*i*) Bactericidal substances, *e.g.,* opsonins, etc. (*ii*) Normal body cells with their phagocytic property, *e.g.,* phagocytes, etc.

The bactericidal action of the blood plays a very important factor. It has been observed by various workers that extravasated blood or blood of freshly-killed animals possesses bactericidal properties which can be destroyed by heating to 56°C. Buchner found that the cell-free serum has the same bactericidal properties as whole blood and he called these bactericidal substances *alexins*. The alexins deteriorate in the presence of oxygen and light and also by keeping. They are either used up or destroyed by contact with living bacteria.

(*b*) Various forms of antibodies produced by the introduction of infective agents or their products into the system.

Apart from these bactericidal substances present normally in the blood various forms of antibodies are also produced from the cells of the system by the introduction of the infective agents or their products. Pfeiffer's phenomenon, hæmolytic immune serum are best illustrations. The properties of natural and artificially produced immune bodies have been studied by various workers notably Bordet, Ehrlich, Morgenroth, Pfeiffer and others. All types of bactericidal serum consist of two substances, (1) thermolabile substance called "substance bactericide" and the other (2) thermostabile substance "substance sensibilisatrice."

The *thermolabile substance* was called *complement* by Ehrlich and Morgenroth and *cytase* by Metchnikoft. Bordet is of opinion that this is identical to Buchner's *alexin*. Bordet and Gengou confirmed by the reaction known as "complement fixation" that this substance (complement) is identical in both normal and immune sera and hence non-specific. The source of the complement in the body is not definitely known. Metchnikoff believed that it is always derived from the living leucocytes. The composition of the complement is also not definitely known. Its sensitiveness to heat suggests that it may be a protein or an enzyme. Hyde has shown that it is a complex substance consisting of at least three portions—the so-called mid-piece the end-piece and a third or heat-resisting component. Dialysis or globulin precipitation, divides it into the globulin fraction (the mid-piece) which fixes itself directly to the cells sensitized or laden with amboceptor and, the albumen fraction (the end-piece) which helps the combination of the cells with the mid-piece. The third component inherited as a simple Mendalian recessive unit reacts with the other two in the combination.

The *thermostabile substance* was called "*amboceptor,*" "*immune body*" or "*antibody*" by Ehrlich and Morgenroth and "*substance sensibilisatrice*" by Bordet and "*substance preventive*" by Metchnikoff. It is regarded to be specific in action and that it is a variable component of the serum and has affinities for the peculiar type of bacteria or red blood cells against which the immunization has been induced either artificially or naturally. Thus a typhoid immune body has no action on cholera vibrio and *vice versa.*

. The immune body acts by combining with its corresponding antigen. Thus if the immune body is mixed with the antigen and kept for some time on ice it can be shown that it has been totally absorbed in the antigen.

Phagocytosis.

Metchnikoff, the father of our present knowledge of phagocytosis, has studied it in numerous types of the animal kingdom and has shown that it is a natural feature of the cell life. It is seen in its physiological condition in the formation of the bone and pathologically by removing the causative organisms, the destructive elements and to produce various forms of antibodies to protect against and to cure the diseases. He attributes this function to two kinds of cellular elements of our body the microphages, and the macrophages. *Microphages* are the polymorpho-nuclear neutrophilic leucocytes of the blood, and occasionally, the eosinophiles and the mast cells. *Macrophages* are the large mononuclear leucocytes of the blood, the "littoral" cells lining the serous membrane and lymph channels, fixed cells of splenic follicles, alveolar lining of the lungs, bone corpuscles, myeloplaxes or giant cells of the bone marrow and the stellate cells of Kupffer in the liver, which Aschoff terms as "reticulo-endothelial system." Metchnikoff further differentiates phagocytic cells into "fixed cells" and "wandering cells." These cells lie dormant in the body in normal conditions showing no activity, except perhaps, the removal of the products of normal cell degeneration, but at once start into vigorous activity as soon as any foreign body is introduced, especially, if it is bacterial in nature. In cases of gonorrhœa, or cerebro-spinal meningitis, it is a striking feature to see a large number of pus cells being packed up with living and often, extremely virulent gonococcus or meningococcus. It was formerly believed, that these phagocytes can devour only those bacteria which are dead and degenerating, but Metchnikoff proved beyond doubt, that they can ingest living and virulent organisms. He demonstrated this fact in case of pigeons inoculated with B. anthracis, in which, the organisms were obtained in pure culture from the leucocytes of the peritoneal exudate.

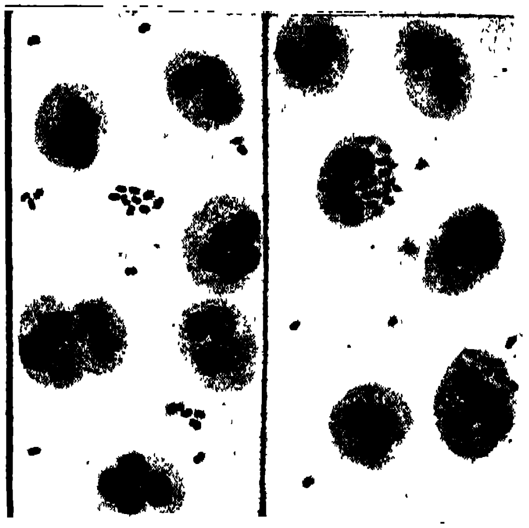
Result of phagocytosis.

The process of phagocytosis is a, more or less, prolonged fight between the phagocytes and the micro-organisms. This fight often continues inside the cells, and in some instances, the bacteria get the upper hand leading to the destruction of the phagocytes. At this stage, the phagocytes may be regarded as somewhat dangerous to the animal, because they afford chances of wider dissemination of the organisms through the cells. In majority of instances, the phagocytes get the upper hand, resulting in intracellular granular degeneration and subsequent death of the organisms. It is generally believed, that the mechanism, by means of which the bacteria are destroyed by the phagocytes, is due to the presence of specific endolysins present in the cells, which Metchnikoff called " cytase "—an unorganised enzyme and is probably identical to what is known as complement or alexin. Metchnikoff differentiates two forms of cytase:—

(*a*) Microcytase.—Acting on bacteria mainly and derived from the polymorphonuclear leucocytes, and

(*b*) Macrocytase.—Acting on cells or animal parasites and derived from mononuclear leucocytes, endothelial cells, etc.

The leucocytes possess no lipase whereas it is present in macrophages. Therefore, the leucocytes cannot digest acid-fast bacilli, *e.g.*, tubercle bacillus. These organisms must be carried to the lymph nodes to be digested by the macrophages.



A B

Fig. 149. Phagocytosis.
A—Bacteria are free.
B—Bacteria ingested by leucocytes.

Opsonins.

It has been observed that although the phagocytic cells are the main agents for the phagocytosis to take place, still, the presence of serum is absolutely necessary. Metchnikoff believed this to be due to the presence of substances in the serum which stimulate the leucocytes to activity, the so-called *stimulus*. Wright then proved that this property is present in the normal serum also, and attributed this phenomenon to be due to substances *which prepare the bacteria for ingestion by the leucocytes, the so-called opsonins.*

Opsonins (from *opsono*—I cook or prepare foods for) are substances present in the normal serum as well as in the immune serum which prepare the bacteria for ingestion by the phagocytes. This has been proved by the following experiment. Bacteria + washed leucocytes + fresh serum = phagocytosis. Opsonins act on bacteria and not on leucocytes. Living bacteria are mixed with fresh serum and kept for some time, it is then centrifugalised repeatedly with normal saline so as to get rid of all serum from the bacterial bodies. The washed bacteria in saline are then exposed to the action of living washed leucocytes when phagocytosis readily occurs. This shows that the bacteria have retained some elements of the serum (opsonins) which have prepared them for the ingestion by the leucocytes.

Normal opsonins are unstable substances, readily destroyed by heating to 60°C or prolonged keeping. They are non-specific. *Immune opsonins* differ from the normal opsonins in their greater stability towards heat and being specific. These immune opsonins are found after immunisation of animals with strepto, pneumo,—staphylo,—meningococci, typhoid, and cholera bacilli, etc. They resemble antibodies of the second order (Ehrlich). The opsonins are supposed to be specific. Thus a man, in a particular period, may have opsonic index with respect to

Staphylococcus	0.82
Streptococcus	0.93
Tubercle bacillus	1.13 and so forth.

Wright has found out a process, by means of which, the amount of opsonins can be estimated quantitatively in a sample of blood, and he determines the amount in the term of opsonic index.

The *opsonic index* is the ratio between the number of organisms of a particular type ingested by a definite number of washed living leucocytes when mixed with patient's serum, and the number of organisms ingested by same number of washed leucocytes when mixed with the serum of a healthy man. Thus in a case suffering from tuberculosis the index (indicated by the Greek letter μ) will be:—

$$\frac{\text{number of T. B. in 100 leucocytes in presence of patient's serum.}}{\text{number of T. B. in 100 leucocytes in presence of normal serum.}}$$

Normal index = 1, ranging from 0.8 to 1.2. (For detailed description see the methods for the estimation of opsonic index.)

The opsonic index is generally below normal in case of acute disease, especially, when it is progressing. It rises as soon as recovery commences, and according to the rapidity of recovery, the rise of the index may be sudden or gradual.

This method is usually used in connection with the diagnosis and treatment of tuberculosis. Too wide a variation of opsonic index (with respect to B. tuberculosis) is always suggestive of tuberculous infection. During treatment with tuberculin, the estimation of opsonic index is of extreme importance. After each injection, there is a fall of the index—the so-called *negative phase,* which, subsequently, rises gradually—the *positive phase*—to normal, and then, higher up due to the production of increased amount of opsonins in blood. When this becomes stationary, another injection is given. This is again followed by a fall which may not be as great as after the first injection. Positive phase commences rapidly, and becomes higher than on the previous occasion. It is important to note, that the negative phase indicates lowering of the immunity rather amounting to susceptibility, and so, any injection during the negative phase, may lead to disastrous results by allowing the rapid spread of the disease and often terminating fatally. It has been observed that the period of negative phase varies with different organisms. Hence it is better not to repeat the injection within that period. The clinical effects—diminution of local, focal or general symptoms, *e.g.,* diminution of fever, pain, etc., may also be used as guides, as they indicate the commencement of the positive phase.

The constitution of the opsonins is not known. Opsonins are abundant in antidiphtheritic serum but there is no bactericidal action. Again, normal and immune sera have strong opsonic action against tubercle bacillus but no bacteriolytic action. These facts show that opsonins are not identical with bacteriolysins. Like complement they are thermolabile.

Chemiotaxis

In an abscess which is full of pus cells, it is a remarkable feature to note the presence of a large number of leucocytes which have emigrated from the blood vessels. This transmigration of leucocytes must take place for the proper phagocytosis to occur. Opsonins render the organisms more palatable to the leucocytes. But what makes this huge number of leucocytes transmigrate? This is generally caused by two factors:—

(*a*) Amœboid movement of the leucocytes allowing them to come out of the blood vessels.

(*b*) The process of chemiotaxis.

The term *Chemiotaxis* means the attraction or repulsion of the leucocytes by means of some chemical agents produced by the micro-organisms (see also diapedesis in the chapter on inflammation).

If a capillary tube, open at both ends, is filled up with a culture of staphylococcus aureus and introduced into the peritoneal cavity of a frog and kept for 24 hours, it will be found (after the period), that the ends of the capillary tubes are filled up with a large number of leucocytes, and many of them have ingested the organisms. Thus, the leucocytes are being attracted to the seat of infection by some process which is known as *positive chemiotaxis.* In other instances, it will be observed that the leucocytes are being repelled from the seat of infection which is known as *negative chemiotaxis;* this, mainly occurs with extremely virulent strains of organism. Certain chemicals, *e.g.*, mercury preparations, turpentine, etc., exert a positive chemiotaxis while quinine, alcohol, chloroform, etc., exert a negative chemiotaxis.

The nature of the substances which are responsible for chemiotaxis is not yet definitely known. It has been found that living bacteria are not always necessary, for chemiotaxis will occur equally with dead organisms, or, even with the products of the bacteria.

Buchner suggests that the chemiotactic properties are due to the products of bacteria which are mainly proteid in nature.

The process of phagocytosis may be summarised as follows:—

1. Hyperæmia of the part bringing in a large number of leucocytes
2. Positive chemiotaxis
3. Amœboid movement of leucocytes.
4. Opsonins

= Phagocytosis = destruction of organisms.

Antibodies produced by introduction of infective agents or their products in the system.

They are:—

(1) Bacteriolysins.
(2) Anti-toxins.
(3) Agglutinins.
(4) Precipitins.
(5) Cytolysins:—
  (*a*) Hæmolysins.
  (*b*) Bacteriolysins.
(6) Antiferments.
(7) Antilipoids.
(8) Albuminolysins.
(9) Bacteriotropins.

1. BACTERIOLYSINS

These are substances produced in the blood, and have the property of causing the dissolution of the corresponding bacteria.

Pfeiffer and his pupils immunised guinea-pig against cholera vibrio, and when they found that the immunity was fully established, a pure culture of cholera vibrio was injected into the peritoneal cavity of the guinea-pig. They examined the peritoneal exudate of that inoculated animal from time to time, and demonstrated, that the organisms had been undergoing remarkable change of shape and ultimately undergoing complete solution, in some instances, within a period of half an hour. They also found that this phenomenon is specific, *i.e.*, the exudate of an animal immunised against cholera will not dissolve typhoid bacilli, and *vice versa*. The phenomena, demonstrated by these experiments are known as *Pfeiffer's phenomena.*

Antibodies of this order are generally produced against cholera vibrio, typhoid and paratyphoid bacilli, B coli, B. dysenteriæ, B. pyocyaneus, B. pestis, etc. It has been observed that the phenomena of bacteriolysis can also be demonstrated *in vitro* with fresh peritoneal exudate. The exudate loses its property if kept for a day or two but the property reappears if fresh serum from a normal (or immunised) animal is added. This shows that the peritoneal exudate consists of two elements—

(1) One destroyed on keeping, but is available in normal or immune serum. This is known as the *complement* or *alexin.*

(2) The other is present only in immunised serum and, not found in normal serum—generally known as *amboceptor* or *immune body.*

Like the agglutination test (Widal reaction), Pfeiffer's phenomena can also be utilized both for identification of the organisms in question, or, the diagnosis of diseases by serological examination.

(*a*) For identification of the organism.—The suspected organism (cholera vibrio?) is obtained in pure culture and injected into the peritoneal cavity of a guinea-pig immunised against cholera. If, after half an hour, the organism shows evidence of disintegration, it indicates that the organism is actually cholera vibrio.

This can be done by suspending the suspected organisms in cholera immune serum and injecting the mixture into the peritoneal cavity of a guinea-pig, rapid bacteriolysis will occur if the organism is really cholera vibrio.

(*b*) In order to search for the corresponding amboceptor in the serum of the patient, the serum is mixed with pure culture of cholera vibrio and made into an emulsion. The emulsion is injected into the peritoneal cavity of guinea-pig. Occurrence of bacteriolysis will show that the serum used contained anti-cholera amboceptor, and thus, proving that the patient has recenty suffered from cholera.

Preparation of anti-bacterial sera—*e.g.*, anti-streptococcic, anti-pneumococcic, anti-meningococcic sera, anti-typhoid, anti-cholera, anti-plague sera, etc.

Type—antistreptococcic serum. A certain strain of streptococcus is isolated in pure culture, and its virulence exalted by repeated passages through guinea-pigs by intraperitoneal injections, until, it becomes so highly virulent that when only a few organisms are introduced subcutaneously to a rabbit it produces fatal septicæmia. This exalted strain of the organism is inoculated subcutaneously into a horse, and afterwards intravenously, until high grade of immunity is produced. The serum of the horse is occasionally tested and standardised during the course of immunisation, and when found of sufficient strength, the horse is bled and serum separated. It has been found, that this serum possesses great anti-bacterial property.

2. ANTITOXINS

Anti-toxins are substances which are antagonistic to toxins. These are generally obtained by inoculating an animal by increasing dose of the toxic products of bacteria. Thus, diphtheria and tetanus bacilli which have got extra-cellular toxins, produce very powerful anti-toxins when inoculated in animals; whereas, other organisms having little or no extra-cellular toxins, will not be suitable for the production of anti-toxins.

Behring demonstrated that when a horse is inoculated with gradually increasing doses of diphtheria or tetanus toxin, it produces a very powerful anti-toxin, which is present in the serum, and can be obtained by separating the serum after bleeding. He observed that this anti-toxic serum will not only protect the human being against infection with the diphtheria bacillus, but, that the disease can be cured, even after it has caused extensive lesions. B. botulinus, abrin, ricin, and snake-venom also produce anti-toxic serum.

Preparation of the anti-toxic serum. This is done in four stages as follows:—

First Stage—*Preparation of the toxin.*—Allow the organism to grow in any suitable liquid medium. When the maximum amount of the toxin is produced, filter the culture through a Chamberland filter. The filtrate contains the toxins.

Second Stage—*Standardisation of the toxin.*—A number of guinea-pigs each weighing 250 grammes are injected subcutaneously with varying amounts of the toxin, until the minimum lethal dose (M. L. D.)—the smallest amount which will kill a guinea-pig of 250 grms. within four days—is determined.

Third Stage—*Production of anti-toxin.*—Small doses (much less than

M. L. D.) of the standardised toxin are injected subcutaneously into a horse, and repeated at definite intervals for a period of two or three months—the injections in the later period being given intravenously. The injection is immediately followed by a fall in the anti-toxin content of the blood only to rise again to a much higher level than before. When sufficiently high degree of anti-toxic property of the serum has reached, the animal is bled, serum separated and preserved by the addition of 0.5 per cent. of carbolic acid, and is ready for use.

FOURTH STAGE—*Standardisation of the anti-toxic serum.*—This is tested against the standard toxin as prepared before and is indicated in the term anti-toxic unit. *Ehrlich's anti-toxic unit* or "immunity unit" is the minimum amount of anti-toxic serum which will neutralise 100 times the minimum lethal dose of the standard toxin when they are mixed together and injected subcutaneously into a guinea-pig weighing 250 grms. and will prevent the death of the animal within four days.

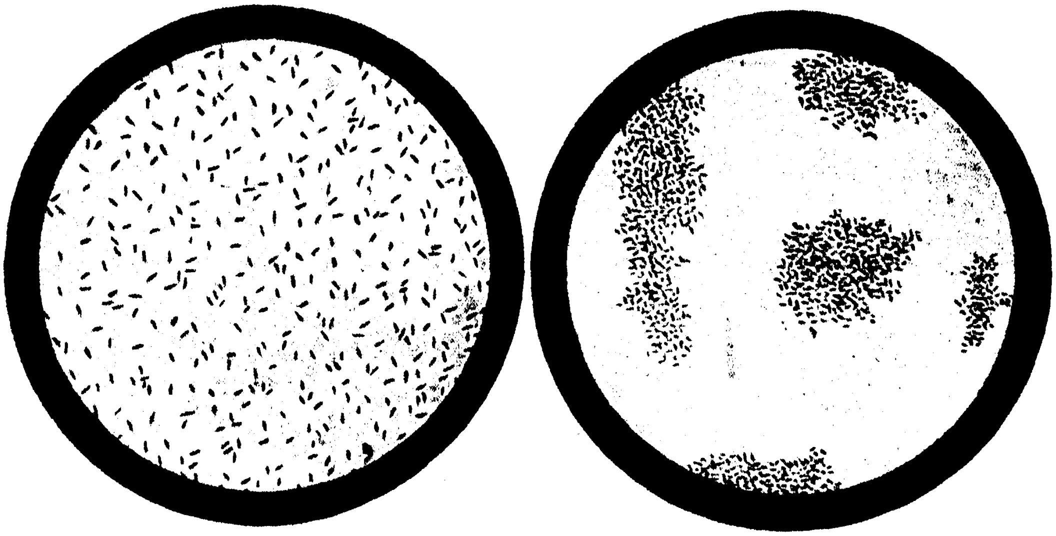
How does the anti-toxin act.

It has been observed that the anti-toxin acts directly upon the toxin, and thus, it is supposed that the anti-toxin destroys the toxin. Morgenroth, in Ehrlich's laboratory, mixed the cholera toxin and anti-toxin and kept for more than a week, thus allowing sufficient time for complete destruction, if any, of the toxin by the anti-toxin. He then treated the toxin-anti-toxin mixture with dilute acids and heated it at 100°C for some time. By this process, he found that the original quantity of the toxin was in its entirety. Calmette has shown that in a mixture of cobra venom and anti-venin the anti-toxin may be destroyed by heat leaving behind the active venom. This definitely proves that the toxin is *not destroyed* by the anti-toxin. Further Buchner showed that the toxin-anti-toxin mixture of B. tetani, which was "neutral" for mice, was still toxic for guinea-pig. Ehrlich expressed his view that a chemical interaction occurs in the toxin-anti-toxin mixture with the disappearance of the specific effect of the toxin as the substance is chemically bound by the anti-toxin. This view is now generally accepted and explained by the Ehrlich's side chain theory. Bordet, on the other hand, regards it not as a chemical combination, but a physical one, corresponding to the process of absorption.

Use of anti-toxic serum—(1) antidiphtheritic serum—6000 units or more (curative), 2000 units (prophylactic); (2) antitetanic serum—500—2000 units (prophylactic) not successful therapeutically; (3) antistreptococcal and antistaphylococcal 25 c. c. (in septicæmia); (4) antidysenteric—25 c. c. (in bacillary dysentery); (5) antivenin—30 c. c. intravenously (specific for cobra and Russel's viper venom, obtainable from Kasauli laboratory).

3. AGGLUTININS

Agglutinins are substances present in serum which cause "clumping" or agglutination and immobilization of the organism when mixed with the emulsion of the bacteria. This reaction is specific, *i.e.*, the serum containing agglutinins against typhoid bacillus will not clump B. coli and *vice versa*. It is practically applied both for diagnosis of the disease as well as for the identification of the organism. If the serum of a man or animal agglutinates B. typhosus, it is suggested that he is suffering or is a convalescent from typhoid, or he may have preventive inoculation thus helping the diagnosis of the disease. On the other hand, if an unknown organism is agglutinated by a known anti-typhoid serum, the unknown organism belongs to the typhoid group of organisms.



Figs. 150. & 151. B typhosus before and after agglutination.

This is generally used for diagnosis of typhoid or allied conditions by the test known as Widal reaction. The agglutination reaction also occurs with dead organisms, and, on the other hand, the agglutinated organisms are not necessarily killed as they have been observed to multiply in the agglutinated state.

This phenomenon is not only found with bacteria, but with other substances as well, and chiefly, with the blood corpuscles. Thus, when an animal is injected with the red corpuscles of another animal, the serum of the former animal is found to possess the specific agglutinins for the corpuscles of the latter. This phenomenon is known as *hæmagglutination.*

The antigen stimulating the production of agglutinins is called the agglutinogen. Agglutinins resist heating to 60°C but is destroyed at 75°C. They are not dialysable. The fact that reagents which precipitate globulin also throws agglutinins, led Widal and Sicard to believe that agglutinins are protein constituents of the serum. Ehrlich termed the agglutinins "anti-bodies of the second order."

Normal, group, and specific agglutinins.

Normal agglutinins are those which exist in the blood of the individual normally and who have not been infected or injected with the bacteria acted upon by these agglutinins. Thus, normal human serum may agglutinate B. coli or typhosus in a dilution of 1 in 5; whereas normal horse serum may agglutinate them in a dilution of 1 in 100 or even 1 in 1,000.

Group agglutinins are those which act on the substances common to the bacteria agglutinated and also the related varieties.

Specific agglutinins are those which act only on the specific components of the stimulating bacteria.

4. PRECIPITINS

These are substances present in serum having the property of precipitating clear filtrates of the corresponding bouillon culture Precipitins are also specific in nature and are not only limited to bacterial products but also to other biological or chemical products. This property is being utilised in determining the origin of blood stains, whether human or not and also used to establish zoological relationship between different animals. Milk, blood serum and other proteins behave in the same way when repeatedly injected into an animal.

5. CYTOLYSINS

These are substances which lead to the solution of cells, either animal, or, vegetable. Thus the term includes—hæmolysins, bacteriolysins, etc. Although they are termed cytolysins, the entire cell rarely undergoes complete dissolution. It is more appropriate to use the term cytotoxin.

(*a*) *Hæmolysins.*—If an animal of some species, say rabbit, be injected with the blood (red blood cells) of an animal of another species, say fowl, the rabbit, after several such injections, will develop some properties in its blood so that when the blood of this rabbit is injected into any fowl, rapid hæmolysis will take place in the fowl.

This phenomenon occurs not only inside the fowl, but, can also be demonstrated outside the body in the test tube. If washed corpuscles of the fowl be mixed with serum of the immunised rabbit, rapid hæmolysis will take place in the test tube. The hæmolytic property of the serum can be destroyed by heating to 60°C for half an hour but can be again restored by adding fresh normal serum which in itself is nonhæmolytic. This proves that the hæmolytic serum consists of two elements, one thermolabile, which is known as *complement*, and the other, thermostabile known as ***amboceptor.*** The process is identical to what we have seen in cases of bacteriolysins.

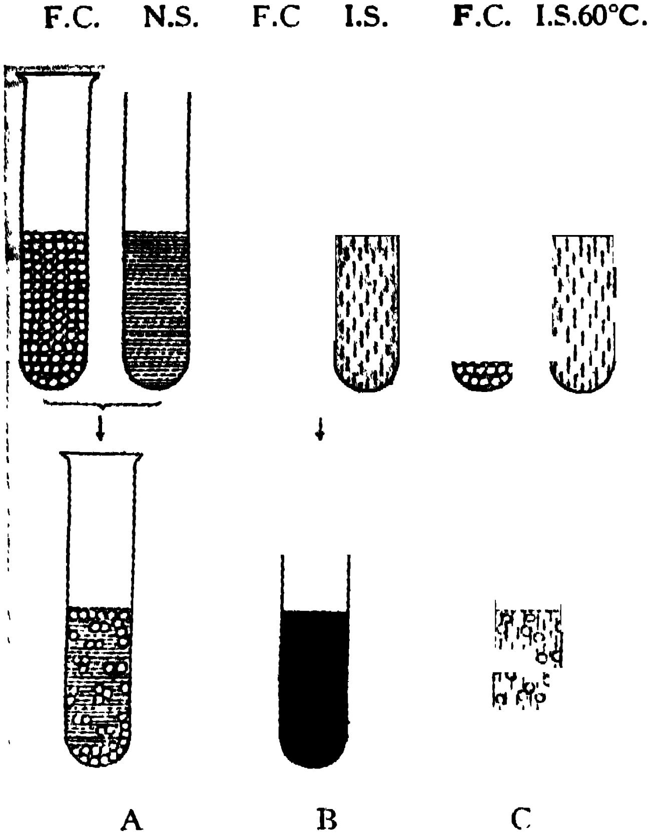


Fig. 152. Showing phenomena of hæmolysis. A.—Fowl's corpuscle+normal serum=No hæmolysis. B.—Fowl's cor-+Immune serum=Hæmolysis. C.—Fowl's corpuscle+Immune serum heated to 60°C =No hæmolysis.

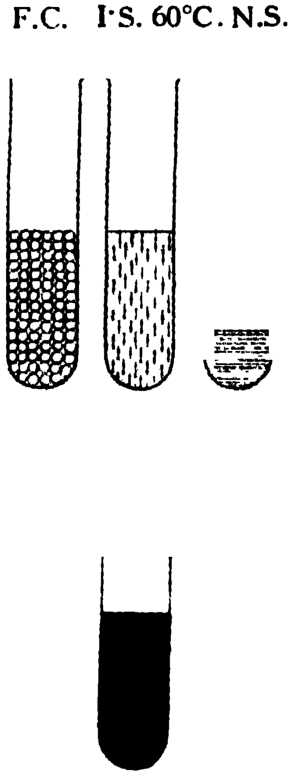


Fig. 153. Showing phenomena of hæmolysis. Fowl's corpuscle+Immune serum heated to 60°C+Normal serum =hæmolysis.

The above facts are summarised thus:—

Normal rabbit's serum+fowl's corpuscles—no hæmolysis.

Immune rabbit's serum+fowl's corpuscles—hæmolysis.

Immune rabbit's serum heated to 60°C and fowl's corpuscles —no hæmolysis.

Immune rabbit's serum heated to 60°C+fowl's corpuscles+ normal rabbit serum—hæmolysis.

From these facts it is concluded that:—

Immune serum represents complement+amboceptor.

Immune serum heated to 60°C represents amboceptor.

Normal rabbit serum represents complement.

(*b*). *Bacteriolysins.—Vide* supra.

Other Cytolysins are—Leukocytolysins, Spermatolysins, Trypanolysins, etc.

6. ANTIFERMENTS

These are anti-bodies with properties to neutralise the corresponding ferments. Antiferments are produced in the serum of an animal immunised against the specific ferments. They are antitrypsin (the antiferment of trypsin), antipepsin (the antiferment of pepsin). Similarly with renin, thrombin, urease, lactase, lipase, etc. The function of these anti-ferments are extremely interesting. We know that the stomach, intestines, and pancreas contain powerful digestive ferments, and the cells of the body, specially the leucocytes, contain powerful proteolytic ferments. How is it that the cells of the body, stomach, intestines, pancreas, etc., are not digesting themselves by the strong digesting ferments present in them? The production of antiferments in the blood—so-called antitryptic contents of blood prevents the auto-digestion of these cells.

7. ANTILIPOIDS OR LIPOIDOPHILIC ANTIBODIES

These are substances present in blood serum of an immune animal possessing the property of reacting with substances belonging to the groups of lipoids. These anti-bodies are found in patients suffering from syphilis, yaws, trypanosoma, leprosy, etc. They have got the power to fix the complement to a greater or less degree from the mixture of the lipoid, antilipoid and complement. This is clinically utilised by the test known as Wassermann reaction.

8. ALBUMINOLYSIS

If, upon a guinea-pig, once injected with normal horse serum or any other protein, another injection is made after an interval of 12 or 14 days—, the second injection is followed by severe symptoms and occasionally death. This phenomenon is known as ***anaphylaxis.*** Thus, the first dose sensitises the animal to a second injection, but certain period of interval must elapse for the anaphylactic symptoms to appear. What these sensitising substances are, it is still not definitely known. They are said to be precipitins in nature, but others suppose them to be some form of lysins—albuminolysins—which produce highly poisonous intermediary albuminoid products causing the symptoms.

9. BACTERIOTROPINS

These are specific antibodies and are not inactivated at 55-60°C. They, like, opsonins, prepare the bacteria for phagocytosis but themselves do not kill them and, they act without complement. They are simple bodies like precipitins and agglutinins and not identical to the bacteriolysins. They are also absorbed by complement fixation serum.

THEORIES OF IMMUNITY

The process of immunity is such a complex subject that scientists are absolutely in the dark as regards the nature of its production. There is no appropriate explanation which will explain all the phenomena of immunity. Three theories, more or less antagonistic to each other, require special study. They are

(1) Cellular theory or theory of phagocytosis of Metchnikoff.
(2) Humoral theory or the side-chain theory of Ehrlich.
(3) Cellulo-Humoral theory.

1. Theory of Phagocytosis.

In the preceding pages we have seen that phagocytosis plays a very important part in the production of immunity. From the fact that the phagocytic cells, like the amœba, engulf and subsequently digest the bacteria, Metchnikoff regards them as the main defence of the body. Although the other processes, *e.g.*, chemiotaxis, opsonins, etc., help a good deal in phagocytosis, still, this phenomena will explain many immunity reactions.

2. Humoral Theory.

This theory assumes that the tissue fluid (as opposed to the cells—phagocytes) contains some chemical substances which lead to the destruction of the invading organisms and their toxins.

That chemical substances, and not, the cells are responsible is proved by the following experiment. Living bacteria are enclosed into a collodion sac and are placed in the peritoneal cavity of an immune animal. The collodion sac will allow only the fluid to enter by osmosis, but not, the cells. When the sac is taken out and examined, the organisms are found to be dead and disintegrated. This shows that degeneration of the organism may take place without the presence of cells.

3. Cellulo-humoral Theory.

It is found, that both the cellular theory and humoral theory will each explain many phenomena of immunity, but none of them are perfect. It has been observed, that bacteria, although not been devoured by leucocytes, still show degenerations. This is more present in places where the organisms are surrounded by large number of leucocytes. This injury to the organisms takes place undoubtedly through the tissue fluid, the chemical substances of which, are supposed to be secreted by the leucocytes. The above experiment demonstrating the destruction of micro-organisms inside collodion sac, can also be explained by the presence of the chemical substances secreted by the cells. Thus, it may be assumed, that the immunity is produced by the chemicals present in the tissue fluid (humoral theory) and that they are produced by the cells, which again, destroy the organisms by ingesting and digesting them (cellular theory). The above theories may be thus combined into cellulo-humoral-theory.

Formation of Antibodies as explained by the Humoral Theory.

(Side Chain Theory.)

Ehrlich assumes that every tissue cell is provided with two functions.

1. The intrinsic function of the respective tissue cell and—
2. The function of absorbing selective nutrient element. This property is selective in this sense that every nutrient particle may not be utilised by all tissues Some tissue prefers some particular nutrient molecule which may be totally rejected by another, and *vice versa.* This selective action is due to the presence of some hypothetical processes called *receptors,* like the limbs of a chemical formula. These receptors will only fix with a corresponding combining process known as *haptophores.*

Similarly, the receptors of a particular tissue cell absorbs nourishment only when the suitable nutrient particle with a similar selective haptophore is presented. One cell may possess receptors of different kinds so as to be able to unite with several haptophores, but the union of receptors takes place only with corresponding haptophores and not with similar ones like the key fitting to the lock.

Ehrlich also supposes that a toxic substance consists of two elements:—

(1) Haptophore which will combine with the corresponding receptor of tissue cells and

(2) Toxophore which will have toxic action on the cell.

A tissue cell, not possessing the corresponding receptor for the haptophore element of the toxin molecule, will not fix the toxin with it, and so, the toxophore element will have no action on the cell.

This is illustrated in case of tetanus toxin. Of all the tissues of the body, nerve cells are supposed to possess the suitable receptors for the haptophores of the tetanus toxin. Thus, when tetanus toxin is introduced into the body, all the tissue cells will refuse to accommodate the toxin, except, the nerve cells.

This is also proved by the fact that when tetanus toxin is emulsified with grey matter of the brain, it loses all its toxicity, and, is absolutely harmless when introduced into an animal. This shows that the toxin has been united with the protoplasm of the brain cells rendering it inert.

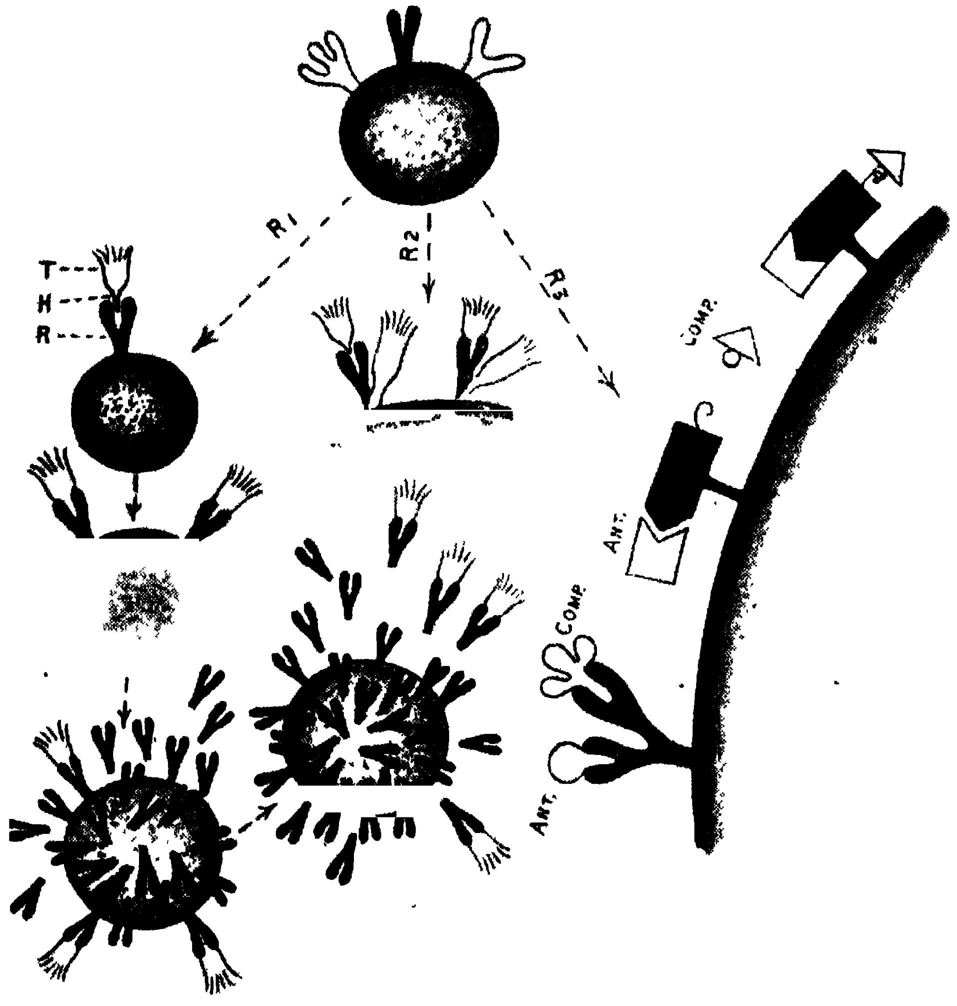


Fig. 154. Diagram illustrating Ehrlich's Side-Chain Theory.

R 1—Receptors of First order. R 2—Receptors of second order. R 3—Receptors of third order. T—Toxophore group. H—haptophore group. R—Receptors. Ant—Antigen. Comp—Complement.

From these facts, the action of a toxin can be explained thus:—

The toxin molecule, on reaching a cell, is either attached to it or repelled, by the presence or absence of suitable receptors. When the receptors unite with the haptophore of the toxin molecule two things happen:—

(*a*) The toxopohore element of the toxin acts on the cell, and,

(*b*) No nutrient molecule can be attached to the cell, as corresponding receptors have already been engaged with haptophores of the toxin molecule. The toxin, thus injures the cell in a double way—by cutting off its nutrition, and, at the same time exerting its toxic effect.

Now, as soon as the cell finds, that the receptors are being engaged with foreign substances and interfering with nutrition, it attempts to produce large number of similar receptors. If another dose of toxin be introduced, some of the newly formed receptors will again be fixed, which, will further stimulate the cell to produce more receptors In this way, by repeated injections, the cell can be trained to produce receptors in increasing number, until, they are so numerous, that the cell is incapable of holding them in its substance and consequently sets them free; and, they then circulate through the system as free receptors. Any further introduction of toxins will at once lead to the fixation of the toxin molecules with the circulating receptors, giving no opportunity to reach the cell and exerting their toxic action. A large amount of toxin—many times the lethal dose—can thus be introduced without the least disturbance to the tissues. This explains the action of immunity after inoculation.

The blood of an animal, thus actively immunised, will contain free receptors, and so, can be introduced into another animal as ready-made immune-bodies which again in its turn will be rendered immune against the toxin thus conferring passive immunity. These forms of receptors are regarded by Ehrlich as—

(*i*) *Receptors of the first order.*—These possess a single combining side chain by means of which they unite with a corresponding group of the antigen. This is what has been already described. This explains the phenomena of the production of antitoxins, antiferments, etc.

The above theory explains nicely the productions of anti-toxins, antiferments, etc., but cannot fully explain the productions of more complex substances, *e.g.*, agglutinins, precipitins, opsonins, bacteriolysins, etc.

In order to explain all these immunity reactions Ehrlich assumes that different kinds of cells possess receptors of three different orders, the detailed study of which is rather complicated and I leave them for special works on immunity.

(*ii*) *Receptors of the second order*—In certain food particles simple fixation of the haptophore group with the similar receptors of cell will not help its nutrition until it is destroyed and assimilated by the cell. This led Ehrlich to assume that the cell possesses two different kinds of receptors, one used for fixing the food particle and the other more or less ferment in character, helps in the assimilation of the food molecule. This, he called *receptors of second order,* which possess one group, to fix the corresponding antigen, and in addition another group (ergophoric group) which leads to a further change to the fixed antigen. This explains the phenomena of the agglutinins and the precipitins. Both the receptors of the first and second orders can lead to the union between the cell and the antigen and to no other substance.

(*iii*) The *receptors of the third order* possess two combining groups one on each side and hence called the *amboceptor* (I stand between). One group like the other receptors fixes the antigen with the cell, while, the other one combines with the complement, leading to the subsequent union between the antigen and antibody. Thus in the process of bacteriolysis Ehrlich regards the combination between the amboceptor and the receptors as a chemical combination but this alone is not sufficient to dissolve bacteria. This is effected by a third element the alexin or complement In this case the complement does not combine with the bacteria nor with the amboceptor rather it combines with the amboceptor after it has united with the bacteria; this final combination produces bacteriolysis. This explains the phenomena of cytolysis, opsonins, and the antilipoids (lipoidphilic antibody of the Wassermann reaction.)—*See* Wassermann reaction.

The theory and practical application of the complement-fixation test.

It has been observed that immune serum or peritoneal exudate consists of two elements—the amboceptor and the complement. (*See* Pfeiffer's phenomenon under Bacteriolysis.)

(*a*) The amboceptor is a component of immune serum, it is relatively heat-resistant (thermostabile) and is specific.

(*b*) The complement is a fragile component of all fresh sera whether normal or immune; it is readily destroyed by heat (thermolabile) and is not specific.

The amboceptor has a strong combining affinity to combine with its antigen. An antigen thus combined with its amboceptor is called to be "sensitized."

The complement will not combine with either the antigen or the amboceptor alone, but when it is mixed with sensitized antigen, lysis of the antigen results and the complement apparently disappears from the mixture. This apparent disappearance of the complement is due to fixation or binding of the complement.

For practical demonstration of this fixation of complement one must use some indicator as the phenomenon is not a visible one. The indicator is usually used in the form of a mixture of hæmolytic amboceptor and the corpuscles used as the antigen for same. This mixture of hæmolytic antigen and hæmolytic amboceptor is called as the "sensitized corpuscles." (Sensitized antigen—*See* above).

ANTIGEN

Antigen is the substance which produces immune reaction (antibodies) when introduced into the animal body. The antigen can be the bacterial toxin, or the bodies of living or dead bacteria, red blood cells, animal cells, chemicals, *e.g.*, proteins, etc. Thus it is seen that the antigen is not necessarily a poisonous thing. Many of the antigens are protein in nature. The antigen is always specific, *i.e.*, one antibody reacts only with the antigen which has produced it and not with any other antigen.

In other instances it may be non-specific. Experimentally it has been found that injection of bacterial vaccine may cause a slight increase in resistance to infection by a heterologous organism. It has also been found that agglutinins are increased by injection of chemical substances, organised ferments (yeasts), inorganic ferments (diastase, pancreatin) or other substances, *e.g.*, nuclein, lecithin even inorganic salts.

ANAPHYLAXIS

Anaphylaxis is the term, as first applied by Richet, used to denote hypersusceptibility or supersensitiveness to the influence of some chemical substances. This is essentially indicative of the condition just opposite to that of immunity and is in contradistinction to prophylaxis. In human being this is generally observed after the second injection of serum. There must be an interval of about 12 days since the last injection. The symptom may arise when the second injection is given even two years after the first. The amount of the second dose does not matter much. A very small amount of the serum after the adequate interval may produce severe reaction while massive doses of serum may be repeated every day for several days together without any untoward result. The symptoms are generally those of heart failure, collapse, dyspnœa, convulsions and rarely even death. Like immunity it may be either natural or acquired, Von Pirquet has introduced the term " protein allergie " to indicate the sensitiveness to foreign proteins. The original dose of the protein which produces this condition is called the " sensitizing dose " and the production of hypersensitiveness is called " sensitization " and the symptoms produced after subsequent injections are described as " anaphylactic shock." Some of the types of allergic conditions are—(*a*) animal proteins *e.g.*, fur of cat, dog, horse and rabbit may produce asthma. (*b*) foodstuffs *e.g.*, shell-fish, strawberries etc. They produce urticaria and diarrhœa. (*c*) drugs, *e.g.*, bromides, salicylic acid etc. (*d*) pollen of certain flowers producing hay fever.

Natural Anaphylaxis

Also termed "Phenomena resembling anaphylaxis."

Some persons are naturally sensitive to certain foodstuffs, *e.g.*, shell fish, cheese, lobster, egg albumen, etc. The intake of these substances or introduction of foreign protein hypodermically often caused serious disturbances in apparently normal individuals.

Asthma following inhalation of the odour of horse, cat, etc., and hay fever due to the inhalation of pollen of some flowers may also be grouped under this group. Drug idiosyncrasy, *e.g.*, with quinine, iodides, salicylic acid, etc., often occur with many persons who are specially sensitive to these drugs with production of symptoms. This hypersensitiveness is in majority of instances primary, *i.e.*, the person never had the drug before.

Acquired Anaphylaxis

(*a*) ACQUIRED BY DISEASE:—

(*i*) As mentioned before—certain infectious diseases notably rheumatic fever, pneumonia, etc., instead of conferring any immunity rather predisposes to a second attack.

(*ii*) The specific reaction called the tuberculin reaction after the introduction of tuberculin as used in von Pirquet and other tests probably belongs to this group. This reaction is found in persons who are suffering from tuberculosis whereas the normal individuals show no reaction at all. This shows the increased susceptibility of tuberculous persons to the introduction of tuberculin. Mallein reaction of glanders is essentially similar to the tuberculin reaction.

Wassermann and Bruck demonstrated the presence of specific substances in tuberculous organs which will combine with tuberculin (tuberculous antigen). This is demonstrated by the complement fixation method. The specific substance present in the tuberculous tissue (antibody) unites with the tuberculin (antigen) with the production of toxic substances which act on the local tissue as well as on other tissues by absorption, thus causing the local, focal and general reactions.

(*b*) PRODUCED ARTIFICIALLY:—

This is exemplified by the following classical experiments—

(*i*) *Richet's Experiments.*—He obtained a toxic substance from the tentacles of actiniæ which when injected intravenously into a dog produced severe toxic manifestations and death in three days. He called the toxic substance "Congestin" on account of the presence of intense congestion of viscera of the inoculated dog examined after death. He further observed, that injection of a sublethal dose of the same toxin produces severe symptoms, but, recovery takes place after a few days. But a second injection into the same animal with a dose 50 times less than the first dose (which would produce no symptom in a normal animal) will lead to fatal result within 24 hours. This shows that the first dose has rendered the tissue hypersensitive to the particular toxin for a subsequent dose which may be a fraction of the first one.

(*ii*) *Arthus Phenomenon.*—Arthus following Richet's line observed that the same phenomena can be obtained with non-toxic substances also, *e.g.*, normal horse serum, milk, white of egg, etc.

He found that when a rabbit is repeatedly injected with normal horse serum a stage was reached when subsequent subcutaneous injections led to the production of marked œdema, severe local reaction even gangrene. If the subsequent injection is given intravenously· severe general symptoms, *e.g.*, convulsions, dyspnœa, etc., set in with fatal termination.

(*iii*) *V. Pirquet's Experiments.*—He found that a certain interval must elapse after the first injection in order that the animal is sensitised so that it responds in the peculiar manner to the second injection. Thus if the second injection is given 5 or 6 days after the first one no symptom will occur, while if given about the 12th day will produce severe symptoms. Thus there is a latent period or *period of incubation* before the development of hypersensitiveness.

In man the presence of hypersensitiveness to horse serum lasts for six months, even longer—5½ years (Allard).

(*iv*) *Theobald Smith Phenomenon.*—He found that guineapigs, injected with toxin-anti-toxin mixture as used in the titration of diphtheria antitoxin, are rendered hypersensitive and succumb to a dose of normal horse serum.

(*v*) *Otto, Roseman, and Anderson's observations.*—

(*a*) They observed that the sensitising agent has nothing to do with toxin or the anti-toxin or the mixture, and that the horse serum is responsible for its production.

(*b*) They further observed that a minute dose of serum 1|10,00,000 c.c. as the first injection is quite sufficient as the sensitising unit. Again a first dose of 10 c.c. into a guinea-pig caused no symptom but the second dose of 0.1 c.c. led to a fatal termination.

(*c*) They found that this reaction was specific, *i.e.*, second injection with the serum from a different animal will produce no symptom.

Passive anaphylaxis

This means that if the serum of an anaphylactic guinea-pig be injected into a normal one, the latter becomes anaphylactic, but unlike active-anaphylaxis this last only for a short time. The anaphylactic property can also be transmitted from the mother to the offspring. When an animal of the same species is rendered hypersensitive, the condition is called *homologous passive anaphylaxis.* When the animal belongs to a different species it is called *heterologous passive anaphylaxis.*

Symptoms of anaphylaxis.

They are restlessness, dyspnœa, feeble pulse, feeble action of the heart, low blood pressure, subnormal temperature and death.

Anti-anaphylaxis or de-sensitization (Besredka).

This is also a very interesting phenomenon. In case of horse serum, the anaphylaxis is generally observed after the 10th day (12-14 days). If the second injection is given before the 10th day, no anaphylaxis will occur, the animal is rather rendered resistant lasting more or less temporarily. This can be made permanent by repeated injections with properly regulated intervals. If, on the other hand, the second injection is required to be given after the 12th day, administration of a very small dose will induce anaphylaxis, but, only in a more or less mild form, which, will again render the animal resistant to a subsequent big dose. Further when a sensitized animal develops anaphylaxis shortly after an injection of homologous protein, the animal remains no longer sensitive to that protein.

Serum sickness. Like guinea-pigs, the susceptibility to an antigen (horse serum) is also found in cases of some human beings which is known as the serum sickness, characterised by fever, urticarial rash, swelling of joints, etc.

Histamin shock. Histamin, present in every complete protein, also produces a symptom-complex in animals almost identical to that of anaphylaxis.

Aggressins. They are substances, endotoxic in nature, produced by the destruction of bacteria which prevent or limit the action of antibodies produced by the host and also paralyse the phagocytic cells but develop immunity when injected into an animal. They are present in the bacteria-free exudates produced in the animal tissue by the organisms. Roux observed that the exudate produced after an injection of the organism of malignant edema produces a marked immunity against this infecton. Bail injected a mixture of tubercle bacillus and sterilized exudate from tuberculous lesion intraperitoneally into guinea-pig which produced sudden death but not if they are injected separately. He assumes that the virulence of the organism is increased by the presence of some substances in the exudate which he termed " the aggressins."

Mechanism of the production of anaphylaxis. The anaphylatic reaction is due to a new toxic substance produced in the blood as a result of the action of anaphylactic antigen (also called anaphylactogen, anaphylaxogen, sensibilinogen, sensitizer, etc.), which produces the hypersensitiveness when introduced into animals with the anaphylactic antibody (also called toxogen, reaction body, albuminolysin, sensibilizin, anaphylactin, etc.,) produced into the body in response to the antigen.

Abderhalden believed that injection of protein into blood produces proteolytic ferments. In anaphylaxis re-injection of the antigen is followed by its digestion by the ferments liberating poisonous products in the blood. Numerous other theories, many based on experimental grounds, have been advocated but the question still remains unsettled.

SHOCK

Shock is a condition of depressed vitality caused generally by injuries. Shock may be primary or secondary. *Primary Shock* is due to the immediate result of the injury. This is generally produced when the patient sees the extent of the injury or the hæmorrhage occurring from it, and may increase progressively when the treatment is delayed. Sudden death may occur from the primary shock, *e.g.*, after severe blow over the epigastrium, or severe peripheral injury directed to the great sympathetic centres in the abdomen, producing reflex inhibition of the heart's action, through involvement of the cardio-inhibitory centre in the medulla. Injury to the testes and small intestines also produces shock. *Secondary Shock* takes place either a considerable time after the primary shock has passed over or independently of the primary one. This is generally due to injuries causing but slight immediate general effects but with subsequent loss of blood, fright or infection.

Causes and Varieties of Shock.—(1) Sight of severe wounds or hæmorrhages on oneself, receipt of bad news, sudden severe fright etc. (2) Surgical shock—severe mechanical injury, *e.g.*, in accidents or in war; extensive rough handling of viscera on the operation table; tearing or dragging of sensory nerves etc. (3) Hæmorrhagic shock

—due to severe hæmorrhage. This may or may not be connected with surgical shock. Loss of one pint of blood in a healthy young man does not produce any shock. (4) Anæsthetic shock—this takes place after administration of ether anæsthesia especially when large quantity is used. (5) Toxic shock—this takes place after injection of toxic substances, *e.g., histamine,* or after absorption of toxic substances liberated from septic processes, *e.g.,* septic peritonitis. (6) Nervous shock or " shell shock"—this takes place mainly due to the nervous strain occurring in modern warfare through the constant noise, horrible sights and excitement. (7) Spinal shock—this takes place after section of the spinal cord and affects only the portion of the body below the level of the lesion in the spinal cord. (8) Gravity shock—this takes place generally in experimental animals, especially rabbits, with pendulous abdomen. When the animal is held in the vertical position with the head above, there is deficiency of blood in the brain due to stagnation of blood in the splanchnic vessels and shock. (9) Cold is also a factor in the production of shock. Generally more than one of the above factors are responsible for the shock. Any single factor may be insufficient for its production.

Symptoms of Shock.—In mild cases there may be only temporary giddiness or fainting, while in severe cases profound disturbance of the circulatory system takes place, with rapid and shallow respiration, subnormal temperature, fall of blood pressure, complete prostration or even death. *In surgical shock,* there are restlessness, pale shrunken countenance, cold sweats on the forehead, great thirst with immediate vomiting if water is given. The skin is cold, moist with sweat, of a peculiar grey colour, lips and gums cyanotic; reflexes greatly diminished not responding until very painful stimulus is applied. Pulse rapid, thin, weak, often imperceptible, respiration shallow and rapid, temperature subnormal, pupils dilated reacting slowly to light. A fall of systolic blood pressure below 90 mm. of Hg. indicates a serious condition. The volume of blood is diminished by loss of fluid from the body through hæmorrhages, sweating etc. The red blood cell count is thus increased in number. A fall of the alkali reserve of the blood takes place resulting in acidosis.

Experimental Production of Shock.—The causative factors may be very easily applied for experimental purposes on laboratory animals specially the rabbit. The following methods are generally applied. (1) The animal is anæsthetised, abdomen opened and the viscera roughly handled especially by pulling and twisting the intestines. (2) The aorta is clamped so that the arterial pressure is kept at about 40 mm. of Hg. (3) The inferior vena cava is clamped off and on for two hours just above the liver. (4) Repeated electric stimuli are given to large afferent nerves. (5) Massive doses of adrenalin injected. (6) By injection of histamine. (7) By crushing the muscles after ligating the limb. The limb is then massaged after removing the ligature so as to allow the crushed products to be absorbed into the circulation. Severe shock results by this method, but no shock takes place if the ligature is not removed after crushing the muscles and the limb is amputated. Injection of the extracts of the crushed muscles or grafting them also produces shock showing that the shock is produced by chemical agencies, probably products of autolysis. (8) By producing extensive burn.

Theories regarding the Mechanism of Shock.—(1) Fall of blood pressure is the most important factor. This is due to a deficient diastolic filling of the heart leading to a diminished quantity of blood being discharged from the heart producing the condition of oligæmia. The fall of blood pressure is not due to dilatation of the arteries or failure of heart, but due to paralytic dilatation of the capillaries producing oligæmia of the functionating tissues. Formerly it was believed that in shock abdominal vessels are dilated. This controversy is now settled. Surgeons invariably find, in severe cases of shock, no congestion of the blood vessels after laparotomy, neither any congestion of the liver either at operation or during post mortem examinations. (2) Crile believes that shock is produced by lowering of peripheral resistance as a result of exhaustion of the tone of the vasoconstrictor centre. It has been proved by various workers that this is not so. Porter and his co-workers have shown that in extreme shock in a rabbit both the pressor and depressor reflexes are normal. It has been definitely settled that the arteries remain constricted during shock. The superficial veins are all contracted. (3) Intoxication by substances derived from the tissues after severe tissue damage is also responsible for shock. This is very evident in experimental animals as well as in war wounds but evidently this is not the main factor in all cases. (4) Formerly it was believed that paralysis of vasomotor centre due to exhaustion, paralysis of the sensory and motor portions of the reflex arc, exhaustion of the suprarenals or acapnœa (loss of carbon dioxide) or fat embolism is responsible for shock. These theories have now been abandoned.

CHAPTER XXIV

FEVER

Fever is a condition characterised by a rise of temperature of the whole body with constitutional symptoms due to disturbances in secretion and absorption. **Pyrexia** generally means only rise of temperature and thus forms only a part of the manifestations of fever.

Fever is generally associated with most of the infectious processes causing inflammation. On account of the frequency of its occurrence with inflammation, it was formerly regarded to be a harmful process. Only during the last few years it has been recognised that fever indicates the response of the body in its defence against the injurious poisons due to the infection. Thus it has been recognised that administration of antipyretics in fever is harmful. It has been found experimentally that agglutinins and bacteriolytic substances are produced in greater quantities and far more quickly in febrile animals than in the control.

Factors in Maintaining Body Temperature.—The average body temperature is 37°C. (98.4°F) with a diurnal variation of about 1°F. The temperature is highest in the late afternoon and lowest during the night, due probably to the greater metabolic activity during the waking hours and upon food intake during this time. The temperature in the mouth is most variable due to talking, mouth breathing, drinking cold liquids etc.

The body temperature is the balance between the heat production and the heat loss. The rise of the body temperature may take place in either of two ways, *viz.*, (1) by increased production of heat and (2) by diminished loss of heat. Heat production takes place in the muscles by the oxidation process of the assimilated products of foodstuffs from the blood. Heat loss takes place mainly through the skin and partly through the lungs. The variation in the temperature of the skin is affected by peripheral vasoconstriction and dilatation and is maintained by radiation, conduction or convection, and by secretion and evaporation of sweat. The regulation of the loss of heat depends to a considerable extent on the general atmospheric condition. Moist air, being a better conductor than dry air, leads to more loss of heat by radiation and conduction but the evaporation of the sweat from the surface of the body is not increased. For this reason in a cold moist day one feels more cold than on a dry day even when the temperature is much lower. Similarly hot moist air causes more discomfort than hot dry atmosphere. In cases of hot moist air with increased heat production due to physical exercise along with very low heat loss, the internal temperature is elevated and condition of heat stroke is produced. This is further favoured in persons possessing a defective vasomotor system. Sunstroke is quite a different affection and results from exposure to the direct rays of the sun, especially over the head.

It is not the increased production of heat which causes fever, but the main factor responsible for its production is the retention of excessive amount of heat. The heat regulating mechanism is kept at a higher pitch and allows the escape of heat only at a higher level.

Causes of Fever.—The chief factors responsible for fever are the toxins of infective processes, injection of foreign protein, hæmorrhage at the base of the brain, action of certain drugs and excessive destruction of protein substances in the body itself.

Types of Fever.—There are generally three types of fever according to the course it takes. (1) Continued or continuous fever is that where the temperature remains at about the same higher level throughout the day with the diurnal variation, never exceeding the normal variation (*i.e.*, 1°F.). (2) Remitting fever—where the diurnal variation is greater than normal but the temperature never comes down to normal. (3) Intermitting fever—where the temperature comes down to normal during 24 hours, generally in the morning, but remains elevated at other times, generally in the evening.

Mechanism of Fever.—*Sudden rise of temperature* produces increased muscular tone with visible contractions in the form of shivering, cutaneous blood vessels are constricted producing chills with pale cold skin, and contraction of cutaneous muscles producing "goose skin." *Sudden fall of temperature* (crisis) produces lowering of the tone of the muscles which become flaccid. Less heat is produced, cutaneous vessels dilated and sweat glands stimulated with marked sweating. *Continuous temperature* is produced by the adjustment of the balance between heat production and heat loss at a higher plane than normal.

Metabolism in Fever.—Basal metabolism is increased specially during the period of chill preceding the fever. In continuous fever the increase in metabolism is proportional to the rise of temperature. Excessive breakdown of proteins, diminished combustion of carbohydrates and excessive breakdown of fat take place when the fever lasts for a long time. Interference with the proper oxidation of the fatty acid molecules leads to the production of acidosis products in urine, and increase in urinary ammonia. The salt elimination is also disturbed and there is retention of salt in the blood. Concentration of chlorides in the blood is diminished.

Changes in the Body during Fever.—There is a specific destruction of proteins in the body. This is shown by the increase of creatinin, or purin bases etc. in urine. This protein destruction probably takes place through (1) high temperature stimulating increased protein metabolism, (2) partial starvation leading to increased destruction of proteins to meet caloric requirements and (3) toxic products directly leading to the breakdown of the proteins.

Symptoms of Fever.—The usual symptoms are rise of temperature (pyrexia), rapid pulse, rapid breathing due to increased CO_2 and high temperature of blood; mouth becomes dry, tongue furred, lips and teeth covered with sordes, appetite and digestion impaired, bowels constipated, urine scanty and high coloured with abundance of urea and urates, general feeling of discomfort and exhaustion. During the stage of rigor blood pressure rises due to constriction of peripheral vessels while the blood pressure falls in the later stages due to dilatation of the vessels. In case of weakness of the heart the pulse is soft and often dicrotic. There are headache, irritation, sleeplessness and other nervous symptoms due to direct action of the toxin on the centre. There may be delirium, specially in children, picking at bed clothes or a semicomatose condition may supervene. In continuous fever the patient may become thin and emaciated.

CHAPTER XXV

INFLAMMATION

"Inflammation" as defined by Sanderson "is the succession of changes which occurs in a living tissue when it is injured, provided the injury is not of such a nature as at once destroys its structure and vitality."

Thus we find, that, in order that inflammation may take place three things are essential, namely—(*i*) The tissue must be living. (*ii*) An injury must be present. (*iii*) The vitality of the tissue must not be totally destroyed.

The object of all inflammation is the prevention or repair of the damage which results from injury. The tissue must protect itself against the invasion of living or non-living foreign substances, and, if already invaded, render the invader harmless which is either metabolised, digested, detoxicated, destroyed or excreted. This function of defence, on the part of the tissue, is manifested by inflammatory reactions. Hence the process of inflammation may be looked upon as Nature's mechanism of defence, which enables the tissue to fight against any form of injury. This mechanism ceases to operate as soon as the injurious agent is removed.

The process of inflammation may well be compared to a defensive warfare which is undertaken to ward off a foreign invasion. In every case of fighting amongst human beings we require soldiers, arms and ammunition to cope with the enemy's forces. The living tissue is not an exception to this rule; the leucocytes are its soldiers, the plasma with the anti-bodies constitute its arms and ammunition, whilst oxygen, red blood corpuscles and serum, supply rations to the fighting force. As soldiers are practically useless in an active fight without arms and ammunition, so the leucocytes are powerless without opsonins and anti-bodies. Besides the soldiers who actually engage themselves in fighting, we require men who will act as messengers by keeping the head-quarters informed as to what is happening in the distant frontiers, and also, men who will act as scavengers by clearing the battle field of the dead bodies. This two-fold function is served by the lymphatics. The first of these functions, they perform by carrying away the dead leucocytes and other cell debris from the inflammatory area.

As the civil population who live in the vicinity of the battle field take but little active part in the fighting, except perhaps the offering of a slight passive resistance, and are chiefly concerned with the reconstruction and rebuilding of the devasted area after the battle is over, so, the tissue cells perform little or no function during the actual process of inflammation, but are mainly concerned with the process of repair of the injured tissue after the inflammation has subsided. It is a well known fact that if the fight is a small one, amounting perhaps to a skirmish, the whole of the country is not informed and it is only perhaps to the neighbouring outposts that the message is sent. Similarly, in the case of the inflammation, if the injury is slight, the reaction also is slight and is scarcely recognized at all. But if, on the other hand, the fight is a big one the news of it is broadcast throughout the length and breadth of the land. Thus, if the injury be severe, and consequently, the inflammation following it be of a severe degree, part or whole of the body takes actual part in the fight, and, as evidence of the excitement that pervades the whole system, there is a corresponding rise of temperature which is often considerable.

Just as, during every warfare, the standing army is re-inforced by fresh recruit, so, the total number of leucocytes in the body (amounting normally from 5000-8000 per c.mm.) is greatly increased, and there is, at the same time, an increase in the production of anti-bodies. Thus, under favourable circumstances, a prompt mobilization of large number of fighting units—the leucocytes, the macrophages of Metchnikoff, etc., and a considerable amount of ammunition—the agglutinins, the bacteriolysins, etc., takes place to fight against the invaders.

In other instances, *e.g.*, in infection with the tubercle bacillus or lepra bacillus, mobilization of large number of other cellular elements mainly those belonging to the group of wandering cells of the connective tissue—the so-called histiocytes may be compared with policemen in the simile—in the form of epitheloid cells, etc., takes place. Not only the soldiers, ammunition, etc., are called upon to defend against the invader, but also other appliances are utilized which offer as barriers—by the exudation of coagulable substances, *e.g.*, fibrin. In diphtheria, for example, the membrane entangles the bacteria and the network of fibrin attempts to absorb the toxin liberated by the organisms.

A battle field, like this, naturally shows extensive destruction of the local tissue and disorganization due to deposition of exudative material, and accumulation of dying, dead and disintegrating bacteria. All these must be removed by the scavengers—the lymphatic system—before the repair can take place.

The concept of inflammation.

This has been admirably discussed by Aschoff.

The following has been taken from one of his lectures:—

"General pathology is nothing more than the study of the morbid affections and of the reactions instituted by the organism to maintain or restore health. It may surprise you that in this classification of the scope of general pathology no place has been set aside for the chapter on inflammation. A great controversy concerning the latter has arisen in Germany and I feel constrained to present to you the salient facts. Considering that so much space is devoted to inflammation in all text books of pathology, it may surprise many that such distinguished pathologists as Andral, Thoma and Ricker advocate that the concept of inflammation be struck from its pages. The well-known hematologist Nægeli once expressed himself as follows: "It is a sure indication of the undeveloped state of a science if the logical concepts with which it deals are not fixed, when multiple meanings are assigned to the same term." In my opinion this statement justly applies to our own field of knowledge with respect to inflammation. We too, assign to it a variety of meanings and as a result controversy arises. The term *Inflammation* (phlogos) was coined more than 2,000 years ago to indicate nothing more than a clinical symptom-complex. Wherever the symptom-complex of a rubor, tumor, calor and dolor was found, it was permissible to speak of inflammation. But not if one or particularly several symptoms of the complex were wanting. How has the concept of inflammation altered since that time? With the resumption of anatomical methods, of investigation and especially with the initiation of histological methods, the clinical symptom-complex was gradually replaced by a morphological one. Inasmuch as the two most important clinical criteria of inflammation, rubor and calor, and especially the relatively unimportant symptom dolor, were no longer present in the corpse, the histologist could study only the inflammatory tumor In the last century controversy raged concerning the latter and the conditions of its development. The phenomena of the inflammatory circulatory disturbances as such were too much neglected even where it was possible to study them in the living animal. Now-a-days we regard the problem of the genesis of the inflammatory tumor as essentially solved. We know that together with the fluid exudation the cellular accumulations play the chief role and that among the latter we can differentiate better than previously the hematogenous wandering cells including the leucocytes and lymphocytes from the histiocytic wandering cells, the histiocytes, the fibroblasts and angioblasts. We know that depending upon the type of infectious agent these various cells can participate in diversified form in the development of the inflammatory tumor. Inasmuch as the alteration in the vascular system, the exudation and emigration generally occupy the most prominent position in all these processes, a few authors believed that this phenomenon was the only suitable criterion of the inflammatory reaction. They asserted that it was not permissible to speak of inflammation in the absence of leucocytic emigration. Thus the conception of inflammation, or rather the symptom-complex of inflammation, was arbitrarily restricted.

On the other hand, many authors regarded the demonstration of active cellular proliferation, *e.g.*, in tubercle formation, as sufficient evidence for the presence of inflammation, although there can be no question here of any well-marked hyperemia or heat production. Indeed we have become accustomed to speak of "cold abscess" in which the most important clinical criterion of inflammation, heat, is absent. It is thus clear that morphological criteria by no means coincide with the clinical ones. As a result the definition of inflammation has become very uncertain. This uncertainty and indefiniteness has become more pronounced with the increasing prominence accorded to the microparasitic causes of the infectious deseases. Inasmuch as the clinical inflammatory phenomena rubor, calor, tumor and dolor on the one hand, and exudatio, emigratio and proliferatio on the other, appear in full-blown form in the infectious diseases, at least in the majority of the latter, clinicians and especially surgeons and gynecologists become accustomed to define inflammatory diseases as infectious diseases, *e.g.*, to identify inflammatory reactions with reactions against infection. It is obvious, therefore, that the surgeon and the gynecologist recognise only an infectious peritonitis following aseptic laparotomy. When they enquire of the pathological anatomist whether signs of peritonitis were found at autopsy, they refer to signs of infection. The pathologist, however, who studies the histological criteria namely, exudation, emigration and proliferation and not the etiology, must admit that every abdominal operation and every surgical wound incision, however, aseptically performed, shows signs of inflammation. It is not infection, however, but trauma which has provoked the reaction observed by the pathologist. These reactions though reparative, show pictures of hyperemia, emigration and proliferation which are very similar to those seen in defensive reactions. The histological findings in the skin about the wound and in the peritoneum necessitate the diagnosis of dermatitis and peritonitis respectively. But these lesions are not necessarily of infectious origin. They may represent merely reparative reactions due to trauma. Similar considerations apply to the inflammatory processes of the knee-joint. There too it is possible to have traumatic inflammation in addition to that caused by infection, the former showing the same clinical signs of inflammation as the latter. Similar illustrations are offered by the brain. In addition to typical abscess formation we recognize atherosclerotic and traumatic foci of softening about which are found reactions of lesser degree but similar in character to those in brain abscess. But the neurologist is just as reluctant to speak of encephalitis or myelitis in the absence of infection as the surgeon or gynecologist is to regard non-infectious reactions of the peritoneum as peritonitis. Similarly, no clinician is willing to designate the organization in a renal infarct as nephritis, although the same morphological signs of inflammation are present.

How can we reconcile these contradictory views? In my opinion there are but two ways. The one is to discard completely the inflammatory concept and speak only of regenerative, reparative and defensive reactions. But the term inflammation is too well established to be eliminated for the present from the physician's vocabulary. If the term is retained, it should be defined neither by the clinical nor the morphological criteria because there are too many contradictions between them. We must seek a clearer definition in order to reach an agreement between the clinician and the morphologist. This is possible only if we choose in place of the purely descriptive definition one which takes into account the significance of the entire process to the organism. I have designated this definition as the *Functional Definition.* If the question is now asked what "inflammation" signifies, it is evident from the choice of the word "inflammation," that the ancients understood by it a special type of excitation and agitation. The term inflammation signifies in a word a state of excitation of the organism. Inasmuch as all reactions, or at least those which are precipitated by material injury, represent a state of excitation of the organism, all materially produced states of excitability may be designed as inflammatory reactions, *i.e.*, inflammation may be identified with a state of reactivity. We would then have to distinguish between regenerative, reparative and defensive inflammation, depending on the exciting cause or the functional effect, which comes to the same thing. According to this nomenclature, the process of regeneration with active cellular proliferation following the section of a nerve would be termed regenerative neuritis, the organization of a renal infarct and the resorptive phenomena following softening of the brain would be designated reparative nephritis and encephalitis, respectively, and the suppuration of the bone marrow resulting from a staphyloccocus infection, defensive osteomyelitis. At any rate, by the addition of the appropriate adjective the clinician can recognize at once what type of inflammatory reaction is meant. Thus the physician will know when he inquires concerning the abdominal operation that a reparative-regenerative peritonitis was found and not a defensive peritonitis."

The phenomena of inflammation. or Histological changes –

This may be studied under three heads—(1) the vascular phenomenon, (2) the lymphatic phenomenon and (3) the tissue changes. We are indebted to Cohnheim who first demonstrated experimentally the phenomenon of inflammation, and since then his experiments have become classical. The process of inflammation may be very easily demonstrated in any transparent living tissue, for which purpose, the mesentery of a frog is commonly used. The mesentery of a frog is spread out on a sheet of perforated cork under the microscope and the various stages of inflammation are directly studied Mere exposure to atmospheric air is quite sufficient to induce inflammation in such a delicate tissue. In such a tissue, we find that the blood vessels are fairly abundant with a continuous current of blood flowing through them; it is almost impossible to differentiate the arteries from the veins and capillaries. In a fairly big vessel, one will notice that the red corpuscles are running in a continuous stream leaving a clear zone in the periphery. This clear zone is mainly composed of serum and is free from all corpuscles excepting a few leucocytes. The rapidity of flow through these vessels depends mainly on their calibre. The flow will be more rapid when the vessels are dilated, and *vice versa.* It will be further noticed that only a small amount of serum is coming out of the vessel, the amount being just sufficient to moisten the serous membrane and the excess, if any, is being very rapidly absorbed through the lymphatics.

When the mesentery is exposed to air or to any other form of injury, these natural findings will be altered to a great extent on account of the severe irritation caused by the injury. A grain of common salt is enough to set up a very violent irritation and start a severe inflammatory process. The leucocytes are seen to come out of the vessel in about 6-8 hours, earliest 3-4 hours and may be as late as 12-15 hours.

1. The vascular phenomena. The immediate effect is a dilatation of the capillaries and venules leading to a rapid and increased flow of blood through them. This stage is called "the stage of hyperæmia or active congestion," and lasts only for a few hours. At this stage, one notices a very curious feature, namely, the appearance of a large number of blood vessels which were invisible before. These are nothing but capillaries which contained practically no corpuscles before, but, which are now rendered prominent on account of the increased amount of circulation through them. The dilatation of the capillaries is followed by a dilatation of the veins, and the rapidity of circulation is gradually replaced by the opposite condition of retardation. This is known as *congestion.* In extreme cases, the retardation may go so far as to lead to a total arrest of circulation, and even to the formation of thrombosis. The dilatation of vessels is caused by either of these factors:—(1) Reflex irritation of the sensory nerve endings by the irritant causing the inflammation. (2) Direct injury to the vessel wall by the irritant, leading to a paralysis of the vasomotor mechanism in the muscular coat of the arterioles.

In rare instances, when the irritation is very severe and sudden, there is an initial contraction of the muscular coat of the arterioles lasting for a very short time which is followed by a subsequent dilatation of the vessels.

With the slowing of the blood current, the corpuscles, which were seen formerly running in the central stream, are found to be scattered all over, and often, come to lie close to the vessel wall. This is purely a physical phenomenon and depends on the velocity of the circulation.

When blood flow becomes sluggish, the leucocytes which are of less specific gravity than the red blood corpuscles, tend first of all to fall out into the peripheral stream. If the rate of flow is still further diminished, the red corpuscles also make their way into the peripheral stream and a mixing of all the elements takes place.

The leucocytes, which normally have no tendency to adhere to the vessel wall, are now seen to come in close contact with it. They also adhere with one another, and this property of adhesiveness leads to a more or less obstruction of the vessels. The stasis forms a vicious cycle, in that, the more the stasis, the more plasma is lost in the form of exudate and the more viscid the blood becomes and this further enhances the process of stasis. The stasis also favours migration of leucocytes towards the wall of the vessel and then into the surrounding tissue.

The stage of stasis is often found to be preceded by a stage of oscillation of the whole current of blood according to the cardiac rhythm, *i.e.*, it is pushed forwards with the systole and dragged backwards with the diastole of the heart, and ultimately, thrombosis may occur. In such an extreme case as thrombosis, there is naturally a complete arrest of circulation, and consequently, proportionately increased damage to the tissue leading to *necrosis, liquefaction and suppuration*. The two main effects of the stasis of circulation are:—(1) Diapedesis—or, the transmigration of the leucocytes and the red blood corpuscles. (2) Exudation. Now we may proceed to discuss each of these effects in detail.

Diapedesis. Strictly means the coming out of the blood vessels of both the leucocytes and the red corpuscles into the surrounding spaces; whereas, *transmigration* is a term used mainly with respect to the leucocytes. If a small vessel, a capillary or a venule, is watched, one will find that the leucocytes are mainly arranged at the periphery; and, if a single leucocyte is carefully observed, it will be seen that a bud of protoplasm is being projected toward the endothelial lining of the vessel and is gradually being pushed into it as a wedge, and then, gradually coming out on the other side. At first, the nucleus remains inside the vessel, but it soon follows the projected mass of protoplasm, the so-called pseudopodia, which is placed partly inside and partly outside the vessel. Soon after, the whole of the leucocyte—nucleus, protoplasm and all are found lying in the perivascular space. The shape of the leucocyte remains irregular throughout the process of extrusion on account of the projections of these irregular pseudopodia. It was formerly believed, that the leucocytes migrate through "the stomata" or the natural openings present in the walls of the blood vessels. The existence of these openings is not accepted now-a-days, and the process of transmigration is attributed entirely to the amœboid movement of the leucocytes, chiefly, the polymorphonuclears. These leucocytes pass out, not through any stomata, but they force their way between the endothelial cells lining vessel-wall, which shows no evidence of rupture. Apart from the amœboid movement of the leucocytes, there is another important factor concerned in the transmigration of leucocytes, and this, is, what is known as chemiotaxis.

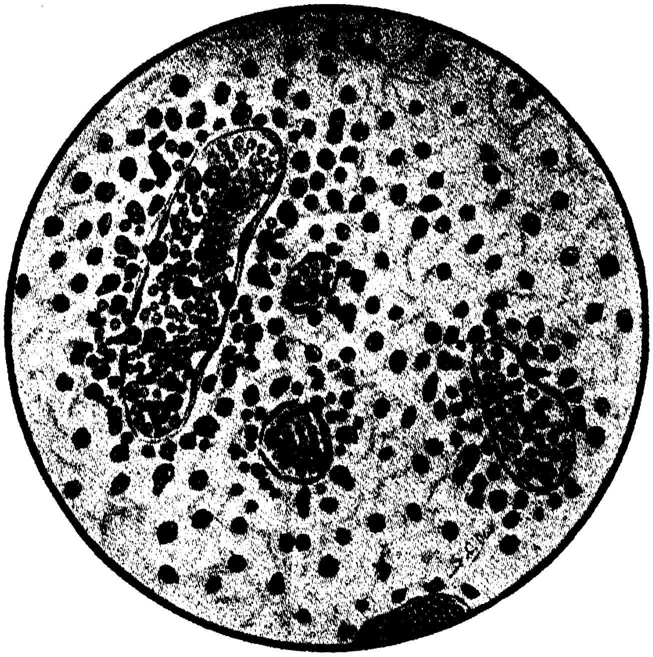


Fig. 155. Dilated blood vessels in acute peritonitis. Note emigration of polymorphonuclear leucocytes and their amœboid character.

By Chemiotaxis we mean, the attraction or repulsion of the leucocytes by means of some chemical agent produced by the micro-organisms.

Thus we find, that there are three factors responsible for the transmigration of leucocytes. 1. Increased permeability of the vessel wall. 2. Rise of pressure within the vessels which presses out the leucocytes (Cohnheim). 3. Chemiotaxis. At the commencement of the inflammatory process there was no cell outside the vessel, but, within a short time, we find that the perivascular spaces are loaded with the leucocytes and red cells. The diapedesis of the red blood corpuscles is entirely due to the intravascular pressure forcing them out of the vessels, after rupture of the over-distended venule. This is also very often due to degenerative conditions of the vessel wall on account of its very frequency in general septic poisoning. Sometimes it happens, that the leucocytes come out in such large numbers, that they occupy all the spaces in the surrounding tissue, exert pressure on the blood vessels themselves leading to malnutrition of the tissue.

Now that we have seen, how the leucocytes migrate from the blood vessels into the surrounding spaces, we many proceed to consider their function. *The function of these migrated leucocytes* are:—

1. They produce a ferment, liberated either during life or after their death, which exerts a deleterious influence on the causative micro-organism. Further some of them break up with the formation of fibrin ferment which causes coagulation of the exudate.

2. They penetrate and infiltrate the inflamed area by the process of positive chemiotaxis.

3. They invade, devour and ultimately digest the micro-organisms within their substances. This phenomenon is known as PHAGOCYTOSIS, and the leucocytes possessing this property are known as *phagocytes.* In certain occasions, *e.g.*, gonorrhœal urethritis, the bacteria appear to multiply within the leucocytes. (*See* also phagocytosis).

4. They engulf, within their substance, dead organisms and cell-debris and thus act as scavengers.

5. Lastly, they form what is known as the ROUND CELLED INFILTRATION, and take part in the repair of the tissues after the inflammation has subsided. Our exact knowledge as regards the phenomenon of inflammation is due to Cohnheim who experimented with chemical irritants only. That inflammation may be caused by bacteria, or their products, led Metchnikoff to study the phenomenon of inflammation with respect to bacterial invasion and came to the conclusion that phagocytosis plays the most important role in inflammation.

Exudation. Along with the cellular elements, namely, the leucocytes and the red blood corpuscles, we find the serum also coming out of the blood vessels and distending the surrounding tissues. The serum with all its albuminous contents, coming in contact with the fibrin ferment produced by the dead leucocytes, become partly coagulated with the deposition of fibrin. Presence of fibrin exudate depends on the tissue affected. It is the rule with all serous-membrane inflammation and acute lobar pneumonia. Certain organisms produce more fibrin while certain other organisms produce more serous exudation. Thus serous exudation is rich in protein and so its presence differentiates the exudative fluid from a transudative one. This accumulation of serum, distending the tissue elements, is known as *inflammatory œdema.* According to the situation, *e.g.,* in cavities—pleural, pericardial, etc., the serum accumulates in the form of free fluid and is known as *effusion.* When it takes place under or in the skin, it forms a *vesicle* or *bleb.*

Forms of exudates—The exudate may be either serous, fibrinous, hæmorrhagic, cellular or, purulent. Serous exudate is produced by mild inflammatory condition. Hæmorrhagic exudate is produced by—(1) Injury to the vessel. (2) Stasis or thrombotic or obstruction of the vessel during inflammation and (3) Increased permeability of the vessel wall or when the vessel is in a diseased condition, *e.g.,* in arteriosclerosis.

The purulent exudate is produced by the accumulation of leucocytes, so extensively, that it becomes opaque, yellowish, thick and generally without fibrin. The pus cells are the poly-morphonuclear neutrophilic leucocytes and contain abundance of glycogen. The purulent exudative inflammation may be—(*i*) Superficial catarrhal, *e.g.,* in gonorrhœa. (*ii*) Circumscribed liquefaction of tissue—(*a*) *Abscess.*—Collection of purulent exudation in a closed non-pre-existing cavity, and (*b*) *Ulcer.*—Superficial defect due to inflammatory and suppurative changes. (*iii*) Superficial, rapidly extending, purulent defect without liquefaction, *e.g.,* phelgmon. (*iv*) *Empyema.*—When collected into a closed pre-existing cavity, *e.g.,* pleura, gall bladder, antrum, etc. (*v*) *Pustule.*—When collected as a superficial bleb.

Mechanism of exudation. It is believed that it is mainly due to the damage to the endothelial lining of the vessels and increased osmotic pressure due to accumulation of the products of inflammation and not due to the secretory activity stimulated by inflammation The functions of the exudate are:—

(1) Mechanical washing away of the irritant causing the inflammation. This is usually beneficial, but may often be dangerous, as the organisms may be carried to the systemic circulation resulting in septicæmia or pyæmia.

(2) Production of the inflammatory œdema, by which the interstices of the tissues are distended, allowing wider space for the leucocytes to emigrate and have full play for their phagocytic action.

(3) Supplying opsonins and other anti-bodies to help the leucocytes in their action.

(4) Supplying of nourishment to the cellular elements taking part in the fight.

(5) Helping the removal of inflammatory products from the area of inflammation through the lymphatic channels.

(6) The fibrin, often, acts as a scaffolding over the inflamed area.

Thus, in the vascular phenomena, we find, all the elements present in the circulation, namely the leucocytes, the red blood corpuscles and the plasma, coming out of the blood vessels and performing useful functions in controlling the inflammation.

II. Lymphatic phenomena. The lymphatic phenomena are secondary to the effects of the vascular phenomena already described. Exudation from the vessels dilate the lymph channels, rendering the tissue œdematous and serving the very useful function of affording enough space for the leucocytes to accumulate and exert their amœboid activity and phagocytic action. The lymph flow is accelerated in these parts; and, the lymph along with the exudate, drain to a large extent, the products of the micro-organisms, the micro-organisms themselves, cell debris, living or dead leucocytes and other elements into the nearest lymphatic gland, which, also may be inflamed due to the presence of the micro-organisms or their toxins. From these lymphatic glands, the materials may be carried ultimately to the general circulation, leading to a systemic infection or intoxication (septicæmia or toxæmia).

III. Part played by the tissues. 1. At the beginning of inflammation the tissue elements, like the civil population of a country cannot take any active part in the fight. But, on account of the large amount of exudation that takes place, the tissues are over-nourished, so that the injury, if it be slight, causes irritation and stimulation of the tissues and ultimately, lead to their proliferation. In extreme cases, however, total destruction of the tissues may result.

Thus, there may be some amount of proliferation in the early stages, but, when the products of inflammation are considerable and those of the micro-organisms destructive, there takes place a widespread (colliquative) necrosis of the tissues, resulting in their liquefaction. This process is known as *suppuration.*

2. The lymph corpuscles accumulate round the blood vessels, and along with the transmigrated leucocytes, form the characteristic round cell infiltration.

3. The endothelial cells, lining the blood vessels and the lymph vessels, are also stimulated like the fixed tissue cells, and, consequently, they undergo proliferation.

The injury causes considerable damage to these endothelial cells, as a result of which, they allow the serum albumin and serum globulin to pass through them. Further, as we have already noticed, the injury also makes the endothelial cells acquire the property of adhering to the neighbouring cells, chiefly the leucocytes, and allowing them to pass through.

The cellular elements found in an acute inflammatory process are the following:—

1. *The hæmatogenous cells,* or those derived from blood and have migrated into the tissue, *e.g.*

(1) The leucocytes, mainly, the polymorphonuclears. Eosinophiles are frequently met with specially in the inflammation of the appendix and the intestines. The polymorphonuclears are identified by the segmented, lobed or polymorphous condition of the nucleus. They are the most constant findings in all acute inflammatory conditions.

They appear within 3 hours after the onset of the inflammation, and if the inflammation subsides, they gradually diminish in number and may according to Beattie, disappear altogether in about 84 to 150 hours. If the inflammation persists they go on increasing. Beattie found by experimental injection of B. coli into guineapig intraperitoneally the following time result:—

"*Non-Fatal cases*:—10—20 minutes.—Very few polymorphonuclear leucocytes found. One hour.—Very slight increase in number,

-2½ hours.—Increase very definite. 3 hours.—A considerable number present. 4½—6 hours.—The increase becoming marked. 6—30 hours.—The increase goes on during these hours. 30—36 and 48 hours.—The numbers begin to diminish. 54 hours.—A very pronounced diminution in numbers. 60—72 hours.—Diminution becomes more marked. 78 hours.—Very few polymorphonuclear leucocytes present. 84—96 hours.—A few still present. They may persist for some days, but from the fifth to the seventh day they entirely disappear.

Fatal cases:—The times at which the various cells appear is practically the same as in non-fatal cases; but the increase in numbers, specially of the polymorphonuclear, is maintained till the death of the animal."

(2) Mast cells. These contain coarse basophilic granules and give a metachromatic reaction with methylene blue.

II. *The Histo-hæmatogenous cells,* or those derived either from the blood stream or from the local tissue cells:—

(3) The lymphocytes or lymph corpuscles—these are either the small lymphocytes migrated from the blood stream or derived from the proliferation of the lymphoid cells which are present normally in the connective tissue, specially round the vessels (perivascular tissue). The lymphocytes and the corpuscles forming the round cell infiltration are characterised by the presence of a fairly large, deeply stained nucleus, almost filling up the whole of the cell. They are more numerous in mild and chronic inflammation.

III. *The Histogenous cells,* or those derived from the tissue cells:—

(4) Endothelial cell lining the serous, lymphatic or vascular channels, contains a big nucleus and a considerable amount of cytoplasm surrounding the nucleus. Like the polymorphonuclear cell, it is phagocytic.

(5) Macrophages.

(6) Plasma cells. These are about the size of large mononuclear leucocytes, with abundance of protoplasm and eccentric nucleus. The nucleus is characterised by the radial arrangement of the chromatin network giving it the appearance of a "cart wheel." The cytoplasm is granular and these granules are specifically stained by Pappenheim—Unna method with methyl green and pyronin.

(7) Giant cells. These are usually foreign body giant cells and

usually found surrounding any foreign body, *e.g.*, sutures, cholesterin crystals, degenerated materials, etc. They can be easily produced by introduction of non-absorbable substances, *e.g.*, horse hair, agar, paraffin, etc.

(8) Other types of cells. These are mainly of mononuclear type present in inflammation and are variously termed by different workers. *e.g.*, polyblast by Maximow (which are amœboid wandering phagocytic cells derived from pre-existing connective tissue, or from pre-existing adventitial cells or even from lymphocytes of blood), clasmatocytes by Ranvier, adventitial cells by Marchand. (See also Reticulo-endothelial system).

Apart from the part played by the local tissue, we find profound changes in remote structures, too. In a case of suppuration, with drainage of pus for a considerable time, one can easily imagine that a huge number of leucocytes are discharged daily and also in a case of pneumonia the number of leucocytes present in the affected portion of the lung may reach to such a figure that it may be many times the total number of leucocytes present normally in the body. This shows that inflammation in a remote part of the body will excite the bone marrow to produce an unlimited supply of leucocytes from the neutrophilic myelocytes. Thus, if during inflammation, the bone marrow is examined it is found to be in a condition of *leucoblastic reaction,* **i.e.*, the fat of the marrow has been greatly reduced and replaced by the myelocytes. In some instances of violent toxic condition, *e.g.*, in small-pox the bone marrow shows occasionally absence of any leucoblastic reaction and even shows degenerative changes of the myelocytes. In such cases there is no leucocytosis rather diminution in the number of leucocytes—*leucopenia.* This is seen in the pneumonias of the alcoholics.

Inflammation of Nonvascular Structure

The classical experiment on the nonvascular structure was on the cornea and that was done by Senftleben. The difficulty of producing inflammation of this structure only, is considerable, on account of, the associated inflammation of the conjunctiva, which obscures to a great extent the changes which are produced as a result of the injury. The cornea consists of numerous parallel laminæ of fibrous tissue separated from one another by numerous freely communicating lymph spaces and flattened connective tissue cells called the corneal corpuscles. Senftleben produced intestitial keratitis with zinc chloride 'solution and found absence of any congestion or diapedesis within 24 hours. The only manifestation was with the corneal corpuscles which showed extensive degenerative condition at the centre of the seat of lesion with evidence of proliferative changes at the peripheral uninjured parts. Only in cases of severe inflammation, polymorphonuclear leucocytes show themselves migrated from the conjunctival vessels. Congestion of the peripheral vessels were seen only in cases of very severe inflammation. The lymph spaces distend enormously due to accumulation of lymph, which. again, is absorbed by the connective tissue cells resulting in œdematous condition of these cells. Repair takes place by extension of capillaries from the periphery to the affected area and replacement of the degenerated structures by proliferation of the corneal corpuscles.

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* Erythroblastic reaction is that in which the fat of the marrow is replaced by erythroblasts in order to produce red blood cells *e.g.*, after hæmorrhage.

The etiology of inflammation.

Inflammation is produced where the tissue is partly damaged by injury, more so when the vitality of the tissue is already lowered.

The etiological factors are grouped as follows:—

I. Predisposing causes.

1. *Local.*—Natural liabilities of certain tissues to damages, *e.g.*, the skin, the mucous membrane, and other surfaces of the body constantly exposed to injury.

2. *General.*—(*a*) Old age. (*b*) Alcoholism. (*c*) Metabolic disorders, *e.g.*, diabetes, nephritis, etc.

II. Exciting causes.

As said above, the injurious causes may be infectious or non-infectious.

1. INFECTIOUS.—Bacteria, fungi, protozoa, worms, insects and other infectious agents of uncertain origin.

2. NON-INFECTIOUS.—(*a*) *Mechanical and Physical.*—Direct or indirect violence, foreign bodies, *e.g.*, gunshot injuries, sprains, burns, scalds, electricity, X-ray, radium and various types of light.

(*b*) *Toxic.*—(1) *Exotoxic.*—(*i*) Organic and inorganic chemicals—*e.g.*, acids, alkalies, chemical irritants, ferments, As, Pb., Hg, alcohol, etc. (*ii*) Animals—*e.g.*, snake venom. (*iii*) Vegetable—*e.g.*, croton oil (2) *Endotoxic.*—In diabetes, gout, nephritis, etc. (3) *Bacterial.*

Clinical manifestation of inflammation. Formerly only four were recognised, namely:—Calor (heat), dolor (pain), rubor (redness) and tumor (swelling). To these, we may now add three more; fever, loss of function and leucocytosis.

We may now consider each of these signs separately.

PAIN.—Pain is usually due to—(1) Stretching of the sensory nerve-endings by the accumulation of the inflammatory exudate after the nerve terminals have been rendered hyperexcitable by hyperæmia. (2) Irritation of the sensory nerve endings by the toxic agents causing the inflammation.

The pain is more evident in those structures which do not yield to the accumulation of the exudate, *e.g.*, the periosteum, fascia, etc. Thus, the more, deep-seated the inflammation, the more, severe is the pain.

HEAT.—Heat is due to active congestion, or, hyperæmia of the inflamed part. Formerly it was regarded to be due to increased production of heat, following increased chemical transportation or combustion. It can be proved by the fact that temperature of the ear of the rabbit can be increased to the same extent as in inflammation (8-10°F) by section of the sympathetic where no chemical factor comes into play.

REDNESS.—This is due to—(1) Active congestion of the part, mainly of the arterioles and capillaries. Although at the commencement of inflammation, the injurious factor irritates the vasodilators (neurotoxic) but finally the opposite condition takes place and from this fact it is settled that the inflammatory hypræmia is entirely due to vasoparalysis (neuroparalytic). (2) Partly to the diapedesis of the red corpuscles.

That it is mainly due to hyperæmia, may be inferred from the fact, that it disappears with pressure. For had it been due chiefly to the extravasation of corpuscles, pressure would not have caused any change.

SWELLING.—This is mainly due to—(1) The accumulation of inflammatory exudate, and partly to (2) the accumulation of cellular elements, (*a*) Derived from blood (hæmocytes), *e.g.*, leucocytes, lymphocytes, etc., and, (*b*) Those derived from tissue cells (histiocytes), *e.g.*, wandering cells from the adventitia of the blood vessels, etc.

Virchow regarded this swelling to be due to increase in the number and size of the fixed tissue cells. The irritative factor causing inflammation produces a form of nutritive and formative irritation to the fixed cells, resulting in the increase in their size and number (hyperassimilation), exudation of fluid, and hyperæmia being of secondary importance.

The discovery of the wandering cells, by von Recklinghausen, who observed them to be present in the adventitia and which can wander when required, has shaken the theory of Virchow to a certain extent but three years later, Cohnheim's epoch-making discovery, placed our knowledge of inflammation on a firm footing. Cohnheim, by his experiments, found the wandering of the cells taking place within three to four hours. He could, further, follow the changes of the vascular system directly under the microscope. (*See* phenomenon of inflammation). This observation went just against the theory of Virchow and Cohnhein asserted, the whole phenomenon of inflammation to be due to vascular changes alone, specially the exudation and the wandering of the leucocytes. All the cells present were taken as leucocytes and the term inflammation came to be used synonymous with leucocyte diapedesis. Subsequently it was observed that a different phenomenon occurs in avascular structures. As for example, in experimental keratitis of pigeons, for the first 24 hours, the main mass of exudate does not consist of leucocytes but of cells of the cornea. Similar observation was also made in transplantation experiments of mitral valve, where leucocyte-like cells were found, evidently due to the proliferation of the fixed tissue cells (c.f. Virchow's theory).

So it is found that the tumour is derived not only from the wandering of movable cells but also due to the proliferation of fixed tissue cells (a combination of the theories of Virchow and Cohnheim). From these points of view, Lubarsch describes "inflammation" as a combined process of alterative and productive changes in the tissues (in the sense of Virchow), with changes of the vascular apparatus with diapedesis of leucocytes (in the sense of Cohnheim). In the avascular structures, the alterative and productive changes come to the forefront but exudative changes, also, do not keep themselves totally absent.

FEVER.—It is not the result of excessive heat production, but of the retention of heat—(1) By the contraction of the cutaneous vessels, and (2) By the cessation of the secretory activity of the sweat glands. Fever, it ought to be remembered, is a reaction which is controlled by the nervous system through the vasomotor mechanism.

LOSS OF FUNCTION.—The pain, swelling, and hyperæmia contribute towards the loss of function of this area. Ordinarily, the tissue is suplied with nourishment just sufficient for its maintenance; but, in an inflamed area, the tissue cells as well as the wandering cells are to be nourished by the amount of circulation present, which often, falls short of the increased demand made upon it leading to nutritional disturbances in the tissues and hence to loss of function.

LEUCOCYTOSIS.—This is the result of stimulation of the bone marrow and other leucopoietic tissue by the inflammatory and bacterial products carried through the circulation into the general system.

Chronic inflammation. Chronic inflammation may be of two types. (*a*) Acute lesion getting into the chronic stage, (*b*) Chronic inflammation from the onset, with a slow progress throughout, and evidently due to very mild continuous irritation. This may be due to—(*i*) Bacterial irritation—tuberculosis, syphilis. (*ii*) Bacterial toxin and other chemicals—cirrhosis of liver. (*iii*) Foreign body—*e.g.*, pneumonokoniosis.

Histologically.—Chronic inflammation is characterised by fibrosis. This is due to overgrowth of the interstitial connective tissue. Sooner or later the newly-formed fibrous tissue contracts, often leading to severe damages. Thus, chronic endocarditis leads to narrowing and thickening of the valves rendering them incompetent to close the orifice. The rigidity of the valves will also cause regurgitation of the flow of blood. In case of diffuse fibrosis throughout the organ, *e.g.*, cirrhosis of liver, the organ becomes greatly reduced in size and the surface becomes granular. In arteriosclerotic kidney there is extensive fibrosis throughout the organ but this is not due to chronic inflammation but due to overgrowth of fibrous tissue in order to fill up the defects left by atrophy and degeneration of the parenchyma of the organ—the so-called *replacement fibrosis.*

Classification of inflammation. Inflammation may be classified as follows:—

According to the Mode of onset

Acute.—When the onest is very rapid. *Chronic.*—When the onset or the course is protracted. *Subacute.*—When the onset is, neither very sudden, nor, very protracted.

Virchow, according to his theory of inflammation as a process of increase in the number and size of fixed cells, classified it, according to the class of tissue affected into (1) *Parenchymatous.*—When the main functionating element of the organ is inflamed. (2) *Interstitial.*—When the supporting connective tissue framework of an organ is affected.

According to the nature of the exudate

(a) Pseudomembranous. This is characterised by the formation of skin-like membranes on the serous or mucous surfaces. It is always composed of necrosed tissue mixed with the exudation products from the blood vessels which is transformed into firm and extensive coagulative material by the action on the dead tissue. Pseudomembranous inflammations can be divided into three groups—

1. *The fibrinous or croupous inflammation.*—This is a very common form of inflammation and takes place generally on the serous and mucous surfaces. Necrosis takes place on the most superficial layers and most often only a part of the epithelial lining is desquamated, so that in many places the epithelium is seen to be present, under the exudate, almost unaffected. The exudation flows out to the surface and is converted into a clot, consisting of fine fibrin filaments by the action of the degenerated surface epithelium. This membrane-like clot can be easily separated from the underlying tissue, so long the process is fresh.

2. *The diphtheritic or pseudodiphtheritic inflammation.*—In this case, the necrosis extends much deeper, so that, many layers of superficial epithelium and even the underlying connective tissue layers are necrosed. The coagulated exudate consists of meshwork of fibrin enclosing leucocytes, red blood cells, and desquamated tissue cells. It is fixed with the underlying structures, and hence cannot be easily separated and a superficial defect is left, when removed forcibly.

3. *Crust forming inflammation.*—In this form the necrosis is most extensive, affecting the whole mucous membrane, including the connective tissue, mucous glands and blood vessels. Thus this necrosed mass, along with the fibrin filaments forms a firm, thick, yellowish or greenish-white crust which cannot be easily detached from the underlying surface. *Example.*—Bacillary dysentery.

(b) Catarrhal. Where the exudate abounds in mucin and the functionating epithelium shows degenerative and desquamative proliferations. Here the exudate from the vessels is mixed with secretion of the mucous glands.

The modern classification is the following:—

(1) The alterative inflammation.
(2) The exudative inflammation.
(3) The productive or proliferative inflammation.

1. The alterative inflammation. In this form there is evidence of sufficient injury causing alterative-degenerative changes of the parenchyma, whereas, there is very little evidence of exudative or reparative processes. It is also essential to note that the exudative and productive changes must not be absent altogether; otherwise it must be classified under degenerative and non-inflammatory condition. This form of inflammation is seen frequently in early stages of nephritis, less so, in other organs and rarely in serous or mucous membrane inflammations. In this form extensive functional disturbances are the prominent features.

2. The exudative inflammation. This is characterised by the presence of exudates, which according to the character is called serous, mucous, hæmorrhagic, purulent or pseudomembranous exudative inflammation. The exit of fluid and cellular elements from the vessels, form the prominent feature. This is most commonly illustrated in case of acute lobar pneumonia. As soon as the acute stage passes off, digestion of the exudates by autolytic ferments takes place restoring the alveoli their normal condition by the process of resolution.

3. The proliferative or productive inflammation. In this form, the defensive and regenerative changes of the fixed tissue cells are the most prominent phenomena to be observed, while the alterative and exudative processes are inconspicuous.

Progress of inflammation. This depends on (1) Nature of the injury causing the inflammation. (2) Period of activity of the injury. (3) Condition of the tissue affected.

Termination of inflammation. 1. Local results *Resolution.*—When the irritation is slight, necessarily the inflammation subsides as soon as the irritant is expelled. The hyperæmia comes to an end, the lymphatics drain away the leucocytes, cell debris and other irritating particles, so that complete resolution of the tissues takes place.

2. *Suppuration.*—When the organisms gain the upperhand the

leucocytes are destroyed in large numbers, the tissue is devitalized by malnutrition and is ultimately liquefied by the enzymes produced by the disintegrated leucocytes and bacterial products.

3. It may pass on to chronic inflammation.

Inflammation of special tissues. *Epithelium.*—When an epithelial cell is inflamed the earliest change is the deposition within its substance, of fine granules, which partly or wholly obliterate the nucleus. The condition is known as *cloudy* or granular degeneration. When the epithelium lines a cavity a large number of cells are *desquamated* (*i.e.*, detached from the basement membrane and cast off).

MUCOUS MEMBRANE.—In case of a mucous membrane inflammation, the most prominent feature will be the *exudation of mucus*, which is produced by the goblet cells. The mucous membrane is red due to hyperæmia and swollen due to the exudation, and the epithelium shows cloudy swelling. The exudated mucus may contain red blood corpuscles and hence may be blood tinged. When the inflammation is of sufficient duration the exudate may be loaded with leucocytes and the colour of such an exudate is yellow. There is generally no exudation of fibrin. When the cavity lined by mucous membrane is very small, as in the case of the appendix, the lumen may be partly or completely obstructed. There is round celled infiltration of the submucosa.

SEROUS MEMBRANE.—Inflammation of a serous membrane corresponds exactly to what we have seen in the mesentery of a frog. Pericarditis, pleuritis (pleurisy), endocarditis, meningitis, are all examples of serous membrane inflammation.

The phenomenon of inflammation affecting serous membranes vary with the nature of the irritant to which the condition is due. There are three varieties of cases (1) so called 'dry' cases in which little exudation occurs (2) those accompanied by free escape of serous or sero-fibrinous effusion and those in which (3) fluid is purulent. Inflammation of a serous membrane is generally the result of the presence of pathogenic organisms which may be admitted either (1) by direct traumatism (penetrating wound, rupture of viscera) or (2) by means of blood stream or lymphatics.

To recapitulate: In the inflammation of a serous membrane, there is hyperæmia of the membrane, followed by diapedesis of red blood cells and leucocytes and finally there is effusion of a serous exudate which rapidly changes its character into a fibrinous one.

There is a limit to the deposition of fibrin and, when that limit is reached, serous exudation only takes place. As the serous membranes nearly always line a cavity the exudate accumulates therein. The amount so accumulated may in extreme cases measure, a few litres. In mild cases the exudation is absorbed leaving the membrane perfectly normal or slightly thickened owing to the accumulation of fibrous tissue. In some cases, however, the two apposed surfaces of the membrane may be found to be adherent to each other by means of fibrous tissue bands. The subserous tissue shows round cell infiltration.

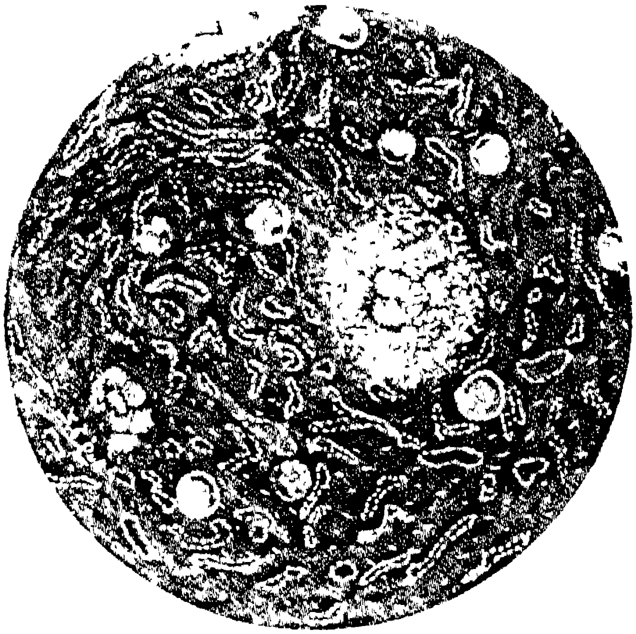

Fig. 156. Abscess in the Kidney.

BONE.—In this case all the vascular phenomena observed elsewhere will no doubt occur but on account of the peculiar situation of the blood vessels within such rigid channels as the Haversian canal, the hyperæmia will be considerably limited. In addition to this the exudation pressing on the blood vessels lead to great nutritional disturbances in the tissue itself which is thereby rendered incompetent to fight the irritant causing the inflammation. Owing to these mechanical disadvantages resulting in early malnutrition of the tissues, they break down and *necrosis* is the result. This happens only when the inflammation is *acute*. In *subacute* cases when the hyperæmia is not so extreme the enzymes produced by the leucocytes and the bacteria cause absorption of the bony tissue resulting in what is known as *caries or osteoporosis*. When the inflammation is a *chronic* one, there is diffuse deposition of fibrous tissue and absorption of calcareous particles leading to a condition of *osteosclerosis*. death

Suppuration. *Suppuration* means colliquative necrosis of an infamed part due to disintegration and digestion of the tissue cells by the products of inflammation.

Causes of Suppuration.—This is due to—I. *Micro-organisms.*—These are mainly of two varieties—(*a*) Pyogenic bacteria, *e.g.*, staphylococcus by producing proteolytic ferments, streptococcus, gonococcus, meningococcus, etc. In case of streptococcus, there is no production of proteolytic ferment in culture and also in cases of highly virulent strain, no pus is formed, *e.g.*, in erysipelas, pneumonia, etc. Pus is formed in less virulent strains.

(*b*) Other organisms—*e.g.*, B. typhosus, B. pyocyaneus, tubercle endotoxin liberated after their death.

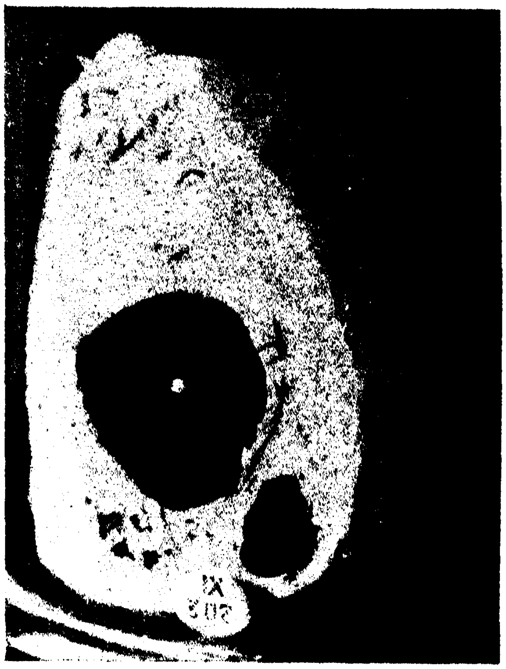

Fig. 157. Liver abscess (front view of the liver). The bigger abscess cavity showing an opening through which it has burst into the inferior vena cava. See also Fig. 158. (From Path. Museum Cal. Med. College).

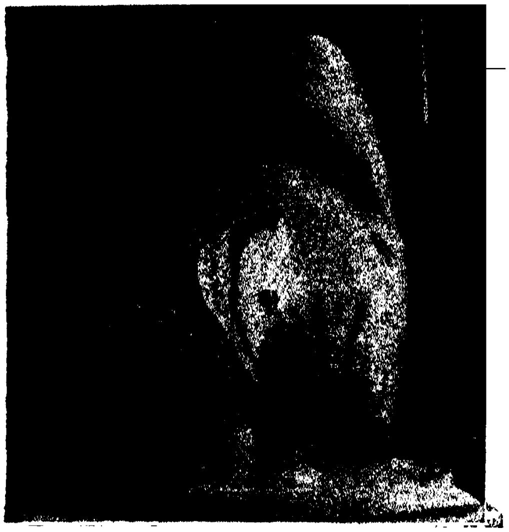

Fig. 158. Liver abscess (back view.) Same specimen as Fig. 157. Showing the opening into the inferior vena cava. (From Path. Museum, Cal. Med. Col.)

II. *Pure aseptic chemicals.*—These are:—(*a*) Pure bacterial protein or their toxin, free from the bacteria themselves. (*b*) Sterile irritants, *e.g.*, turpentine, croton oil, etc.

Suppuration, due to chemicals, is always local and can never produce metastasis in the form of pyæmia.

As the leucocytes accumulate in enormous numbers in an inflamed area, a large amount of available nutrition is used up by them. This leads to a condition of malnutrition of the tissues, which consequently undergo dissolution or colliquation, with the formation of a more or less circumscribed cavity filled up with cell debris, blood stained fluid and dead or degenerated leucocytes. Such a cavity is called an *abscess* and it contains *pus*. Pus consists of large number of cells—the pus corpuscles and the fluid element—liquor puris. The fluid has a specific gravity of about 1030 and is rich in proteins. It also contains products of proteolytic digestion. Occasionally red blood cells are also present in pus. The process of pus formation is known as suppuration. The tendency of all abscesses is to travel along the path of least resistance and to evacuate either externally or into internal cavities with the escape of purulent material. The track communicating the abscess cavity with its point of bursting is known as the *sinus* and if at the sametime it communicates with a hollow organ of the body (*e.g*, the intestines) it is called a *fistula*.

Plate No. VI

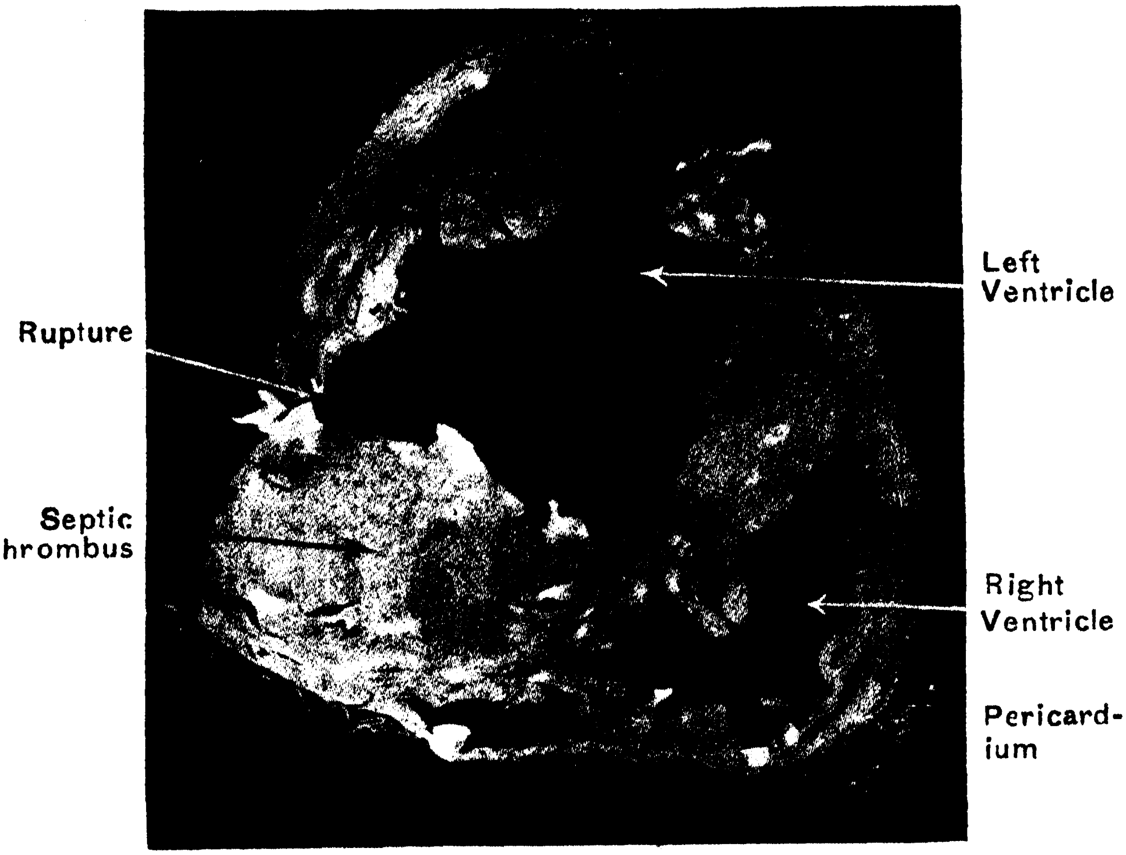

Fig. 159. Total frozen section of heart showing rupture of the left Ventricle following Suppurative myocarditis.

The abscess may be either single or multiple. It may be a localised one, but if the infectious material is carried through either blood or lymph stream into the general circulation, the formation of multiple or pyæmic abscesses results.

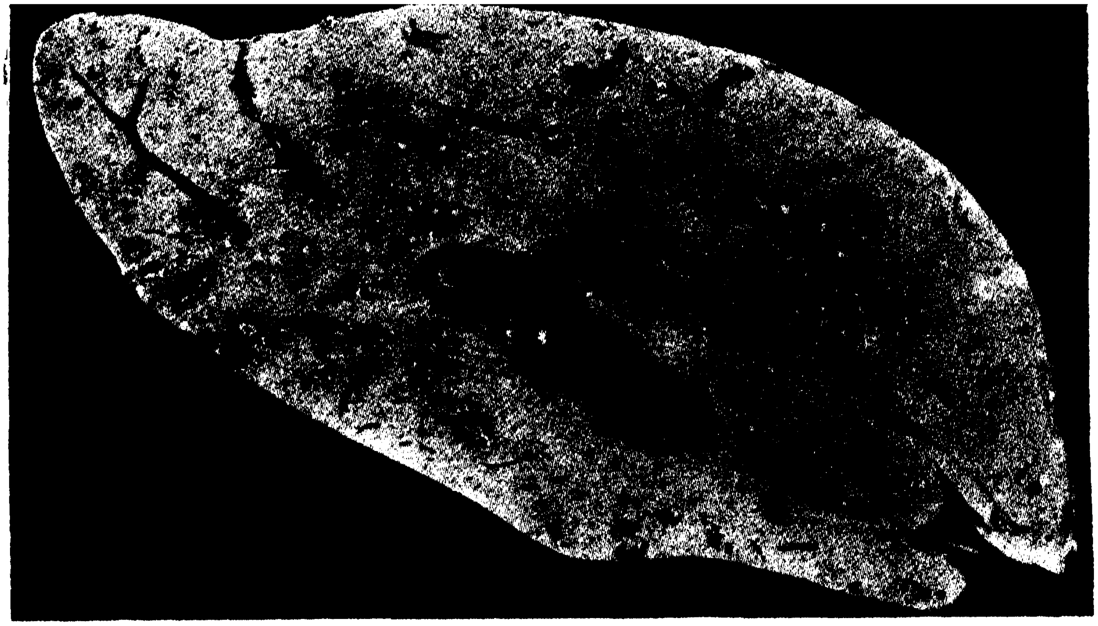

Fig. 160. Total frozen section of multiple abscess of the Liver.

When a small abscess is seen under the microscope, one notices in the centre a homogeneous purulent material with distintegrated tissue cells surrounded by a large number of leucocytes with practically no tissue cells. As we go to the periphery the leucocytes and the round cell infiltration become less and less and we find an increased proliferation of the tissue cells.

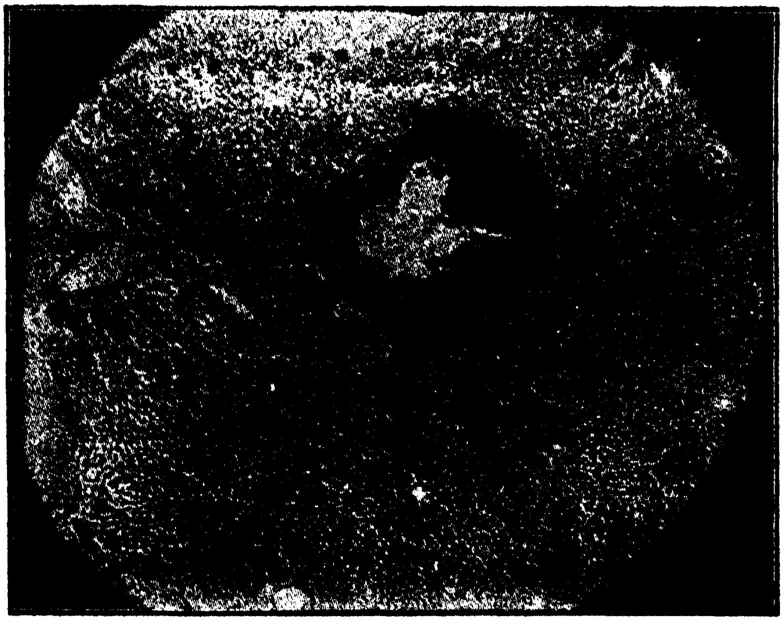

Fig. 161. Abscess in the liver, showing necrosis in the centre surrounded by masses of leucocytes.

CHAPTER XXVI

REGENERATIVE TISSUE CHANGES

With the subsidence of the inflammation the destructive processes are controlled and the reparative changes commence in order to make good the loss sustained by the tissues, unless they are devitalised too much by the injury. The changes are essentially the same in nearly all the tissues irrespective of their situation, there being only slight modifications due to local conditions. All reparative processes ultimately lead to the production of fibro-connective tissue which unites the divided structures and fills up the gap formed by the necrosed material.

The favourable regeneration depends upon the following factors —(*a*) close approximation of the divided segments, (*b*) rest to the part, (*c*) presence of asepsis, and (*d*) the extent of damage already suffered by the tissue.

The Healing Process

Although regeneration depends on the above factors, the underlying principle is the same in every case, and slight modifications are seen under different conditions. Whenever there is loss of continuity of a tissue or even when mucous or other surfaces are involved, the purpose of regeneration is to remove or absorb the exudates, cell debris, etc. and replace the lost tissues by the proliferation of connective tissue corpuscles and endothelium lining the capillaries.

The process of healing is generally classified as follows—(1) healing by primary union; (2) healing by first intention; (3) healing by second intention or granulation; (4) healing under a scab; (5) healing by the organization of a blood clot, or an inflammatory exudate; and (6) healing of the individual structures.

(1) *Healing by Primary Union.*—This occurs when the two surfaces are in apposition without any other structure intervening: direct union takes place of the blood vessels and other cellular elements of the two segments. This is only theoretical, because, however mild may be the injury, there is some amount of exudation or extravasated blood clot between the two segments interfering with the primary union.

(2) *Healing by First Intention.*—This occurs in clean-cut aseptic wounds where there is no loss of tissue in the form of a bruise.

There is no collection of blood or discharge excepting a thin layer of blood clot and a microscopical zone of inflammation between the two segments. The union takes place through the thin layer of blood clot which is ultimately absorbed. The union usually takes place in about 5-7 days.

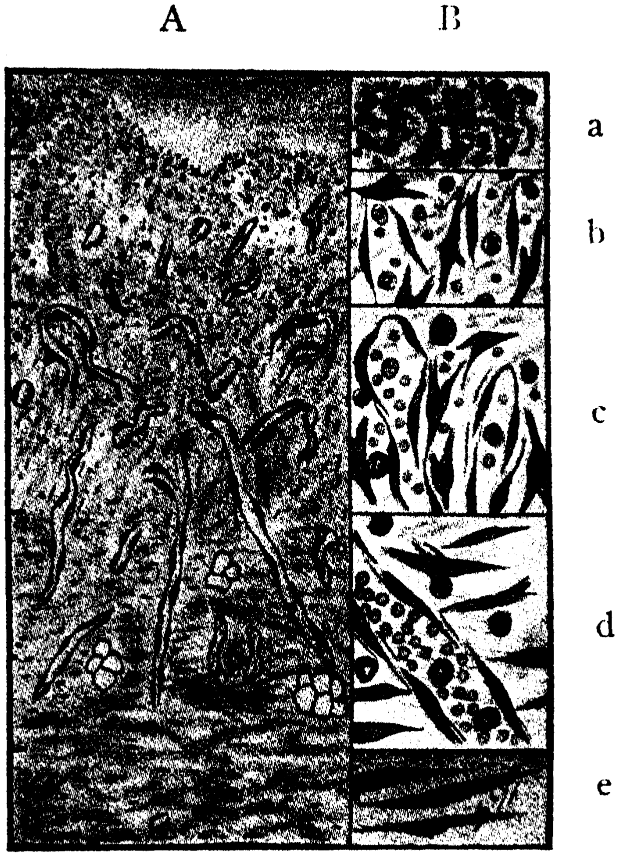

Fig. **162.** Structure of granulation tissue.

A—Under low magnification.
B—Under high magnification.
a—Surface layer shewing leucocytes and red blood cells.
b—Few newly formed blood vessels.
c—Numerous newly formed blood vessels with capillary loops. Large number of fibroblasts mainly running parallel with the vessels.
d—Fibroblasts nearly at right angles to the vessels.
e—Normal healthy tissue.

(3) *Healing by Second Intention or Granulation.*—This takes place when (*a*) there is a definite loss of substance, (b) the two segments are not in proper apposition, (*c*) there is some amount of inflammatory product intervening with rapid healing.

No healing takes place until all the infective material and the dead tissues are removed. This work of scavenging is done by the leucocytes which disintegrate and absorb the dead material. In a case where there has been surgical interference, the dressing also partly carry on the work of removal by draining the purulent and other discharges, leaving the wound free from all irritation. Presence of any slough will keep up the irritation and will interfere with the formation of granulation tissue. The granulation tissue originates primarily by the formation of a large number of new capillaries at the bottom of the wound. This is usually effected by the proliferation of the endothelial cells lining the exposed capillaries. This overgrowth of the endothelium of the capillaries leads to the production of solid masses of protoplasm which gradually increase in size. Very soon they unite with one another and with similar masses produced by the damage and subsequent proliferation of the endothelium of the neighbouring capillaries, so that still larger masses are produced.

After a time these protoplasmic masses (masses of endothelial cells) become channelled by the blood stream penetrating into them from the original blood vessels. Thus loops of capillaries are formed which are in direct communication with the original blood vessels. Sooner or later, either by the irritation of the dressings or otherwise, some of these capillary loops rupture with the further proliferation of the endothelial cells which are again canalized with the formation of new capillary loops.

Thus we see that a tissue growing from the bottom gradually fills up the cavity. This tissue is mainly composed of a large number of endothelial cells, the pre-existent round cell infiltration and a vast number of capillaries. These cellular elements grow in number and size and on account of their mutual pressure they become flattened, with the formation of spindle cells, which in their turn become more and more flattened, with the formation of fibroblasts. These fibroblasts begin to grow usually from the periphery connecting the granulation tissue with healthy structures in the neighbourhood.

The Histological Picture of Granulation Tissue consists of—(*a*) Large number of newly formed capillary blood vessels with very thin walls. (*b*) Uninucleated endothelial or epitheloid cells. (*c*) Uninucleated wandering cells—macrophages (Metchnikoff), plasma cells (Unna), polyblast (Maximo), clasmatocytes (Ranvier), adventitial cells (Marchand), etc. (*d*) Uninucleated round cells. (*e*) Polymorphonucleated leucocytes—in cases of persistent inflammatory process. (*f*) Multinucleated giant cells. (*g*) Fibroblasts.

In course of time when the whole cavity is filled up with a large number of these fibroblasts they begin to contract, at the same time pressing upon and finally obliterating a large number of formed capillaries. The proliferations of the cellular elements are put a stop to with the development of fibrillar connective tissue. This connective tissue contains enough of collagen fibrils but few elastic fibres and no nerve fibres. The deposition of collagen material separates one cell more and more away from the other, and so the granulation tissue once containing very large number of cells and vessels very soon changes into a fibro-connective tissue, poor in cells and vessels. Thus the granulation tissue which was formerly a very vascular structure is gradually converted into an avascular scar tissue which contracts very soon. The retraction of scar tissue is not only seen in the skin but also in internal organs, *e.g.*, kidney, spleen, liver, etc. In certain cases there is an exuberant growth of granulation tissu coming out of the surface which is known as an *exuberant granulation.*

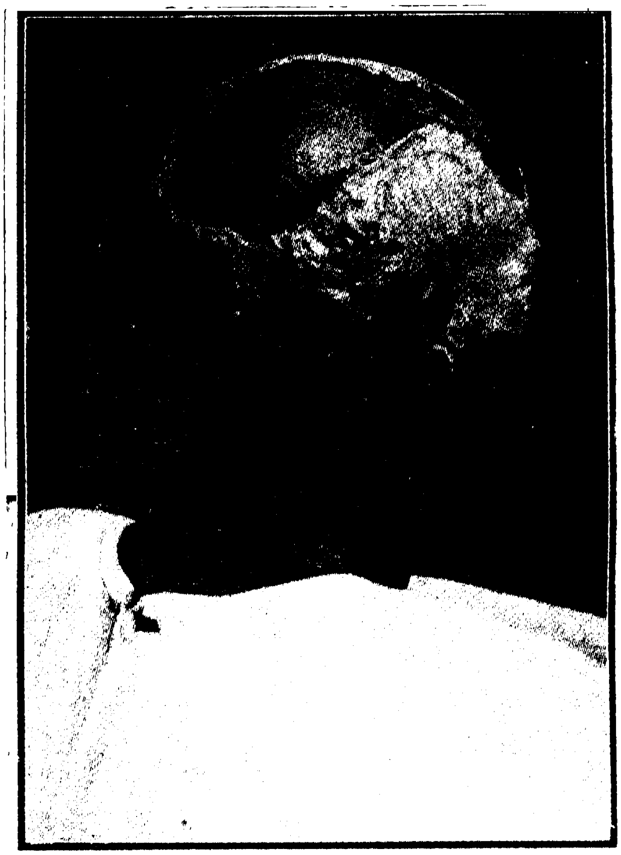

Fig. 163. Extensive ulceration of the scalp.

(4) *Healing under a Scab.*—This is possible only when the wound is a small one, situated superficially and is free from all sepsis. The inflammatory exudate dries up or the extravasated blood is clotted with the formation of scab. The healing takes place by granulation, as in the previous case, underneath the scab which protects it from injury and infection. In case there is an infection present, the purulent material is apt to collect under the scab, interfering with the healing process.

(5) *Healing in a Blood Clot and Exudate.*—Here a blood clot or an exudate acts as a passive agent, which is infiltrated by leucocytes and serves the purpose of a support for the granulation tissue, which gradually replaces the clot or the exudate. These can be best studied in cases of subcutaneous effusion of blood or in fibrinous pericarditis.

Healing of Special Structures

Epithelium.—(a) Surface epithelium regenerates rapidly by the multiplication of cells adjacent to the wound. The newly formed epithelium gradually spreads over the granulating area and thus controls its exuberant production. Any advent of micro-organisms, denudation of the surface or other accidents will interrupt its growth. (*b*) Glandular epithelium, *e.g.*, mammary gland. It is not certain whether the destroyed mammary tissue can regenerate to its full extent. It is replaced to a large extent by fibrous tissue. In the case of liver cells, newly formed bile capillaries often try to take up the function of liver cells, in addition to actual proliferation of the existing liver cells. (*c*) Columnar cells often regenerate to a considerable extent. The epithelial lining of the bronchus may regenerate completely even after loss of considerable amount of tissue in cases of acute bronchitis. Similar is the case of the urinary tubules.

Muscular Tissue.—The striped muscle fibres can regenerate by multiplication of its cells but non-striped muscle fibres are mainly replaced by connective tissue. For example, in the case of wounds of the uterus, repair takes place by fibrous tissue formation. The same thing takes place in the case of cardiac muscles.

Elastic Tissue.—Regeneration of elastic tissue takes place to a considerable extent. This is generally derived from the pre-existing elastic fibres in the tissue. It takes a long time to regenerate and when the new tissue is formed its function and appearance are not perfectly normal.

Tendons.—These can regenerate to a certain extent.

Bones.—Bones regenerate by the formation of new bony tissue by the transformation of the granulation tissue cells into osteoblasts and deposition of calcareous particles in the intercellular substance. This is known as the *callus.* It differs from true bony tissue by the absence of the Haversian systems and is known as *provisional callus.* The provisional callus is formed much in excess of what is necessary, the excess being subsequently absorbed, leaving what is known as *definitive callus* which remains permanently.

Blood Vessels.—These regenerate to a considerable extent, as we have seen in the case of granulation tissue, and unless they are compressed by fibrous tissue this regeneration is permanent.

Cartilage.—In this case, regeneration is impossible; repair takes place by fibrosis.

Nervous Tissue.—The highly specialised nervous tissue, *e.g.*, the ganglion cells, do not regenerate, but the less specialised tissue, such as the peripheral nerve fibres, do regenerate to a considerable extent.

CHAPTER XXVII

SPECIFIC GRANULOMA

Specific granulomata are inflammatory conditions resulting in a more or less subacute or chronic course with the formation of granulation tissue. Unlike the granulation tissue produced by septic organisms the specific granulomas are caused by some particular micro-organism with special peculiarities in each case.

TUBERCULOSIS

Tuberculosis is a specific infectious disease caused by the bacillus tuberculosis and characterized by the formation of nodules called tubercles. A tubercle is an inflammatory, more or less circumscribed nodule which undergoes degeneration in the form of caseation, necrosis and ulceration or heals with the formation of fibrous tissue and subsequent calcification.

The term "Tuberculosis."—The course and progress of tuberculosis can be divided both pathologically and clinically into different periods, each having quite distinct and peculiar manifestations, of which the tubercle-formation is a particular phase. From this fact Aschoff is of opinion that it is not justifiable to call these affections "tuberculous" as it implies only one period of the disease, *i.e.*, that of tubercle-formation. This he mentions, in comparison, "one might as well call syphilis gumma-disease." Aschoff suggests that "it was only gradually, especially since the discovery of the exciting agent by Koch who named this organism the tubercle bacillus, that the fashion of employing the word tuberculosis, instead of phthisis, came into general use. It is about time that we should again employ the name phthisis for this disease-entity. Then we shall see that tuberculosis is only a special reaction form in the course of phthisis."

History.—Our first real knowledge of tuberculosis dates from 1819 when Lænnec observed the nature of the tubercles, detailed the physical signs caused by them and interpreted their mutual relation and significance. Villemin in 1865 showed that, on inoculating tuberculous material into a rabbit, miliary tuberculosis was produced, proving the infectious nature of the disease. In 1882 Koch discovered the causative organisms and proved their pathogenicity.

Tuberculosis is a widespread disease in the animal kingdom. Man, domestic animals, even wild animals are subject to the disease.

Cattle are the commonest among the domestic animals to be infected. Monkeys usually suffer when confined. In human beings one-seventh of all deaths are due to tuberculosis and 40-50 per cent. of deaths from other diseases show evidence of tuberculosis post mortem; Naegali says, about 90 per cent. Aschoff (Germany) is of opinion that more than 90 per cent. of all living beings have a primary infection, of whom about two-thirds develop a re-infection. Of these reinfected individuals half get absolutely well. It is present in all races and all climates. The mortality from tuberculosis is rising in India and falling in England.

Age Incidence in Tuberculosis.—It is rare under one year; after the fifth year it is more frequent, and most frequent between twenty and thirty.

Sites.—(1) Lungs.—This is the commonest situation, being affected in about 95 per cent. of cases, either primarily or secondarily, from some other focus. (2) Serous Membranes, *e.g.*, pleura, peritoneum, pericardium, meninges, etc.—Pericardium is said to be rarely affected in India, but this is not the case. (3) Lymphatic Glands.—Tuberculosis has special predilection for cervical, tracheo-bronchial and mesenteric glands. (4) Organs.—Intestines, larynx, liver, kidney, spleen, brain, etc. (5) Bones and Joints. (6) Fibrous tissue and muscles.—These are very rarely affected. (7) General.—This is usually known as *acute miliary tuberculosis*. In this condition the organism circulates in the blood and is carried to distant parts, causing either localised lesions, *e.g.*, tuberculous meningitis or arthritis or general miliary tuberculosis.

In adults, the lungs are the commonest situations of the disease, whereas, in children, glands, bones and joints are usually affected.

Modes of Infection.—(1) *By Hereditary Transmission.*—This may be through—(*a*) spermatozoa—no undoubted case on record; (*b*) ovum—possible; (*c*) placenta—Boyd has mentioned that tubercle bacilli have been found in the mesenteric glands of an infant one day old, and also in a still-born infant. These cases are usually accompanied by placental lesions. Warthin and Cowie have pointed out the fact that fœtal blood may contain a large number of tubercle bacilli without any lesion of the tissues.

(2) *By Inoculation.*—In this condition the disease is nearly always local and is commonly found in butchers, taxidermists, veterinary surgeons and those who perform post-mortem examinations. The infection takes place by the inoculation of tuberculous material of

the dead animal through some abrasions present on the skin of the person infected. In case of butchers a wart-like growth is produced which is commonly known as *butchers' wart* or *verruca necrogenica*.

(3) *By Inhalation*.—This is supposed to be the commonest mode of infection. The dried sputum of a tuberculous patient containing a large number of tubercle bacilli, when inhaled by a healthy individual, may reproduce the disease in him. Cornet's view is that fine particles of dried sputum mixed with dust are the source of infection. On the other hand Flugge is of opinion that moisture is essential and hence the particles of sputum must be in a moist state in order that they may give rise to infection: it is the moist particles of sputum floating in air that are harmful agents.

That infection takes place mainly through inhalation is proved by the facts—that (*a*) the lungs are the organs commonly affected; and (*b*) persons in close contact with tuberculous patients are affected more than others.

Recently Calmette's experiments showed that the particles of dust do not reach the lungs by inhalation. The ciliated epithelial lining of the upper respiratory passages sweeps out all dust particles which are thereby prevented from reaching the alveoli. He further proved that carbon particles introduced into the stomach reaches the lungs, causing anthracosis. The fact that carbon particles are normally all taken up by inhalation and not by any other route and that they actually reach the lungs is against Calmette's hypothesis. Moreover, the tubercle bacillus may be taken up, from wherever it may be arrested, by a phagocyte and carried to the lymphoid tissue or lymph node, more specially from the fact that tuberculosis is essentially a disease of the lymphoid tissue although the function of this particular tissue is to destroy the invading organisms. It may also be possible that, irrespective of the original seat of lesion, the tubercle bacillus may be carried from this focus through the lymph channels ultimately into the thoracic duct, subclavian vein, right heart and pulmonary artery into the lungs, establishing a tuberculous focus in the lung. This is very nicely illustrated in case of experimental tuberculosis in guinea-pig by subcutaneous or intracutaneous inoculation into the thigh. These theories are still under discussion, but the general evidence is in favour of the inhalation theory.

The organisms reaching the alveolus are taken up by the lymphatics into the inter-alveolar spaces where they produce the inflammatory or degenerative changes, resulting in the production of a tubercle. In a certain percentage of cases, the larynx is affected secondarily from pulmonary tuberculosis, which is commoner in Europe (10 per cent.) than in India (3 per cent.).

(4) *By Ingestion.*—The tuberculous material is taken up with the infected food material, commonly through milk. The organisms are either taken up by the tonsillar mucous membrane or are allowed to pass into the intestines. The organisms can enter the body through either intact or abraided mucous membranes. From the tonsils they are carried by means of the lymphatics to the cervical lymph glands, causing cervical adenitis. These glands become infected one after another and either burst externally or may infect the tracheo-bronchial glands and ultimately even the lungs themselves. From the intestinal mucous membrane, either intact or abraided, the organisms enter into the mesenteric glands, causing what is known as tabes mesenterica and subsequently peritonitis. Thus the sequence of events are—Tuberculous ulcer of the intestines—Tabes mesenterica—Tuberculous peritonitis.

When the organisms enter through intact intestinal mucous membrane they may cause primary tabes mesenterica without producing any intestinal lesion. Again the organisms have been known to pass unopposed through the intact mucous membrane as well as through the glands, producing primary tuberculous peritonitis. From the peritoneum the organisms may pass upwards through the lymphatics and may infect the bronchial glands or the lungs.

The organisms causing pulmonary tuberculosis are usually of the human type whereas those causing intestinal lesions are commonly of the bovine type. Bovine tubercle bacilli are more virulent for the cattle than the human type of bacilli and the former are commonly found in affections of glands, bones and joints. In India 10 per cent. cases are of bovine origin whereas the figure is as high as 20 to 30 per cent. and even 50 per cent. in England.

Bovine type of infection has been experimentally produced by feeding monkeys with milk from infected cattle and also with meat of infected animals.

Natural infection of cattle is commoner in Europe than in India. Pigs are also found to be infected. In some cases the sputum of a tuberculous patient is swallowed by himself, giving rise to intestinal lesions of the human type.

In the intestines the areas richly supplied by lymphatics are commonly affected. Although the gastric juice does not kill the tubercle bacilli, still the infection of the stomach is rare. Of the small intestines, the lower part which is richly supplied with lymphatics as is evidenced by the presence of Peyer's patches and solitary follicles, is the part that is readily affected. Tuberculous lesions of the large intestines are not so common as those of the lower portion of the small gut (*cf.* dysenteric ulcers which are found in the large intestines only).

(5) *Through Blood.*—In cases where the bronchial glands are affected caseation takes place with adhesions of the neighbouring structures including the blood vessels. If the wall of one of these vessels is eroded the caseous material is poured into and carried through the circulation into distant parts of the body. If one of the bronchial arteries is affected the organisms are carried to a part or whole of the lungs causing acute pulmonary miliary tuberculosis. In case the caseous matter is evacuated into the pulmonary vein it is carried throughout the body, causing acute general miliary tuberculosis with or without localisation (*e.g.* Tuberculous meningitis). Unlike typhoid or other organisms, the tubercle bacillus does not grow while circulating in the blood but it is transported from place to place by means of the blood current giving rise to local or general lesions. Thus the lung acts both as the receiving and a broadcasting station for the tubercle bacillus.

The spread of the lesion within the lung may take place in one or more of three ways—(1) by the lymphatics, the commonest method producing nodular groups of tubercle; (2) bronchogenic *i.e.*, through aspiration of tuberculous material from a necrosed area into healthy portions forming tuberculous bronchopneumonia; and (3) through involvement of the lumen of the pulmonary artery by the lesion and transmitting into other parts, producing what is known as the miliary tuberculosis.

Acute Miliary Tuberculosis.—This condition is characterised by the presence of a large number of small tuberculous nodules, all being more or less of the same age and so of the same character, due to sudden extensive dissemination of the tubercle bacillus throughout the lungs (acute pulmonary miliary tuberculosis), or throughout the whole body (acute general miliary tuberculosis). This is always secondary to a primary focus. In cases where the primary focus is not easily detected, a minute search of the pulmonary vessels or of the thoracic duct will invariably show the lesion affecting the lumen of the vessel—the so-called *intimal tubercle.*

Miliary tuberculosis indicates that the tissue finds sufficient time to produce local reaction inspite of apparently very heavy blood invasion. This shows that the tissue must have attained a certain amount of immunity beforehand due to some primary focus, otherwise the heavy invasion would have caused death.

The presence of isolated tuberculous lesions in remote organs as bone, kidneys, Fallopian tubes, meninges, etc., at once suggest that the tuberculous infection must have reached the organs through the blood stream. The probable source is that bacilli from an active focus are drained by the lymphatics into the great vessels and thence are discharged into the blood stream. As the number of the organisms thus drained are much small, the lesions are naturally localised. But, on the other hand, if the discharge is heavy, as in case of a rupture of a caseated hilar gland into the pulmonary artery, the lungs, according to the distribution of the pulmonary artery involved, will show a large number of miliary nodules, all more or less of same size and appearance. This constitutes *acute pulmonary miliary tuberculosis.* If the material is discharged into the pulmonary vein, the bacilli will pass through the left heart and aorta and will be distributed throughout the whole body, forming *acute general miliary tuberculosis.* In case where the tuberculous nodules are not of same size, they generally form more than one set or groups of nodules, each set or group being of same size. This is due to more than one crop of invasion of the tuberculous seeds through the blood stream from some primary source.

Pathogenicity.—All cultures of tubercle bacillus are not pathogenic to man nor to all animals; this is due to the existence of several different strains of the organism. The bovine strain plays a very minor role in the production of the human pulmonary tuberculosis. All human tuberculous lesions of the lungs are due to the human type. The affections of the bones and joints, lymph nodes, the so-called surgical types of human tuberculosis and also many cases of tuberculous meningitis and general miliary tuberculosis of children are generally caused by the bovine type. It is a common fact that both types are rarely found together: thus, the occurrence of pulmonary tuberculosis and surgical tuberculosis at the same time is rare. The immunity produced by one type protects the individual from infection with the other: thus, a child getting a mild dose of infection (the immunising dose) of bovine type through drinking milk, may develop healing or latent lesions which will protect the child against a much bigger dose (the destructive dose) of human type of bacilli. Bacilli of human type may not infect the cattle and *vice versa.* Again, the question of tissue resistance plays a very important part in the role of infection, some tissues being more vulnerable to infection than others. This is what Osler termed " good or bad soil."

Primary Infection and Re-infection.—It was formerly believed that the tuberculous condition of the adult is the result of further manifestation of the original infection acquired in childhood and thus endogenous in nature. The present conception is that the active tuberculosis of the adult is the result of re-infection from outside. The primary infection generally takes place in the lungs and may occur in various parts, but the sites of predilection are the upper lobes, especially of the right lung. This primary infection is usually single and lies invariably immediately under the pleura and possesses a connective tissue capsule separating it off sharply from the surrounding lung tissue. There may be a caseous focus in the adjacent lymph node. This is generally found between the ages of 8 and 14 years. It is, in contrast to the re-infection, a single focus. There is a group of miliary tubercles surrounding the primary nodule. A chain of miliary nodules may often be observed along the lymphatics to the lymph nodes. The primary lesion undergoes caseation, but cavity formation does not take place. In both the primary focus, which is of the nature of exudative-caseative bronchopneumonic focus and the gland, formation of specific granulation tissue takes place rapidly transfering them into fibrous scar tissue. Deposition of calcareous particles are frequent. In a majority of instances the primary infection heals completely leaving only a scar which may be calcified or even ossified. Opie has found a very ingenious method of detecting these calcified nodules of healed pulmonary tuberculosis post mortem by means of X-ray films taken of the lungs after removal from the body.

The lesions of re-infection are usually multiple and generally affect the apex of the lungs, not at the extreme apex but about one inch below the extreme tip, less frequently other portions and are situated more within the substance than subpleurally. When the lower lobe of a lung is affected, the lesion, again, starts about one inch below the actual apex of that lobe. The right lung is affected more frequently than the left one, probably due to the right bronchus being more directly continuous with the trachea. Healing takes place by the production of fibrosis. The lymphatics are blocked early so that the tuberculous material rarely reaches the regional lymph nodes as also the carbon and dust particles, rendering the tuberculous focus more pigmented. Calcification of the caseous material may also take place. *Endogeneous Re-infection* is that due to metastasis in the lungs alone or other parts of the body from tuberculous material derived from a primary focus already present in the same individual. *Exogeneous Re-infection* is that due to infection of the lung, already the seat of tuberculosis, with tuberculous material from further external source. Metchnikoff and others have shown that the primary infection generally takes place in the childhood (mostly during first five years of life), which leads to immunization of the individual so that the re-infection in the adult, as a result of fresh sufficiently massive infection, breaking down the immunity leads to the, formation of fibrocaseous type with cavity formation, while the lesion of the primary infection is more caseous. People far away from the environments of modern civilization show, as a rule, most serious and virulent type of infection, with massive caseous pneumonia and absence of any fibrosis, as they are not immunized by the primary infection occurring in infancy.

This gives three different forms of lesions in tuberculosis: (1) focal tuberculosis of children, (2) healed or latent tuberculosis of the adolescents and adults, and (3) pulmonary tuberculosis of the adults. This was illustrated in the World War. In the Western Front "the South African Labour Corps, consisting of Kaffirs and Cape boys, had more deaths from tuberculosis than had the whole of the British troops in France. In 1918 the Kaffirs numbered 11,000 with 113 deaths, the British numbered 1,500,000 with only 74 deaths."—(Boyd.) These facts regarding the effective primary immunising dose and the adult destructive dose in human tuberculous infection, have prompted Calmette and Guerin to bring out the B. C. G. vaccine (Bacillus-Calmette-Guerin vaccine). This consists of a living culture of tubercle bacillus which has been kept in artificial media for several years, thus attenuating to a great extent but still retaining its antigenic power. The injection of this attenuated organism in large doses in susceptible animals produces no disease. Since 1921, they are using this vaccine per mouth on infants, brought up by tuberculous parents, during the first ten days of their lives. The result on human beings are not yet settled.

Lesions.—The tubercle bacilli always enter the body through lymph channels and produce lesions in those places where their onward progress is arrested. Thus, in the lungs they are taken up by the lymphatics and then pass from the alveolus into the interalveolar septum where the endothelial cells lining the lymph channels offer resistance. From the lymphoid tissue of the lung the organisms gain access to the lymph nodes at the root. The involvement of the lymph nodes differs to a considerable extent in the child and the adult, due mainly to anatomical grounds. In the child the lymphoid tissue of the lung is of "open" type, *i.e.*, the lymph cells are few in number, the sinuses wide and dilated, offering a ready passage to the bacilli to be drained to the hilar lymph nodes. In the adult, on the other hand, the constant accumulation of the irritating dust particles, with the passage of years, leads to hyperplasia of the lymphoid tissue leading to narrowing and even blocking of the lymphatic sinuses and thus offering great resistance to the passage of the organisms. This arrest of the bacilli explains why massive tuberculosis in the adult lung may be associated with slight involvement of the hilar nodes. Thus, in the child, the tuberculous affection is more pronounced at the roots of the lungs than in the lungs themselves while in the adult the reverse is the rule. Thus the involvement of the regional nodes is characteristic of primary rather than of secondary tuberculous infection.

Owing to the irritation caused by the bacilli the endothelial cells are stimulated so that they multiply rapidly and surround the organisms. These endothelial cells are not derived from the alveolar epithelium. They are derived from histiocytes of the reticulo-endothelial system. They are highly phagocytic and are variously termed as dust cells, heart failure cells, foam cells, etc. As the stage advances, the zone of endothelial cells spreads outwards and at the same time a large number of lymph corpuscles from the lymph channels or the perivascular lymphatic space accumulate there with the formation of what is called round cell infiltration. No new-formed capillaries appear in the tubercle thus formed. The tubercle is thus a non-vascular structure. Owing to the destructive action of the products of the bacteria, as well as to the obstruction of circulation resulting from partial or complete obstruction of the blood vessels supplying the part (endarteritis obliterans), the central part of the focus suffers from loss of nourishment and consequently undergoes coagulation necrosis. The necrosed material is cheesy in character, hence the process is termed *caseation*. Calcareous material may be deposited in the caseated areas. The caseation is more common in glands and often occurs in the kidneys. In case of the vertebræ, the caseous masses often attract phagocytic leucocytes chemiotactically and form a cheesy pus, which when in considerable amount, burrows along the muscles of the back. This process is not accompanied by any signs of acute inflammation, and so this is called *cold abscess* and according to the seat of the lesion, *lumbar or psoas abscess*. As the process is nearly always sub-acute or chronic, regenerative process proceeds simultaneously in the peripheral part of the focus and well developed fibrous tissue is sometimes seen even in early stages. It sometimes happens that the endothelial cells when unable to resist the organisms become converted into giant cells.

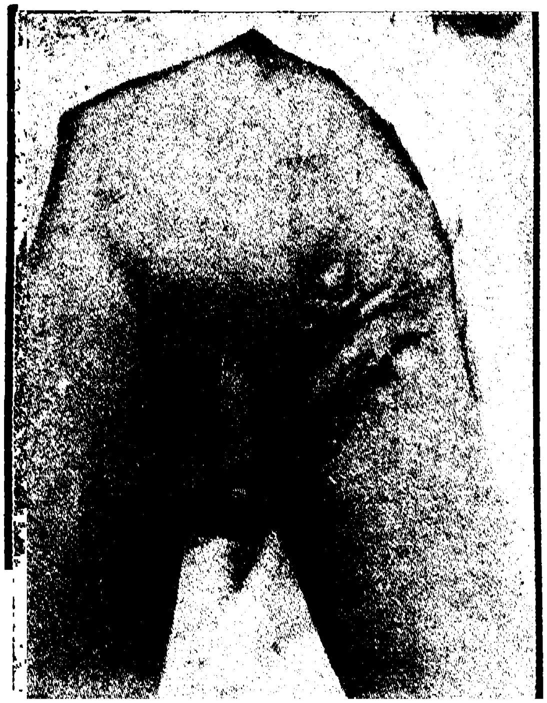

Fig. 164. Granulomatous ulcer of the groin.

How the giant cells are formed is not exactly known. It is believed that during the rapid multiplication of endothelial cells it occasionally happens that the nucleus divides very rapidly without giving sufficient time for the cell to divide; the result is that a big cell is formed containing many nuclei. Others are of opinion that giant cell formation is a degenerative process which takes place by the fusion of several endothelial cells, the nuclei remaining separate with the formation of a big cell with several nuclei.

Of these two different views the latter seems to be more acceptable for the following reasons: (1) The centre of the giant cell itself shows necrotic areas. (2) The nuclei are arranged peripherally suggesting that the nutrient matter is present mainly at the periphery of the giant cell. (3) Endothelial cells surrounding the giant cells are often found in degenerative conditions.

The succession of changes mentioned above due to the activity of the tubercle bacillus results in the formation of a small nodule, varying in size from a pin point to a pin head, which is known as a tubercle. The tubercle is the initial lesion in all tuberculous* processes.

Thus in the case of tuberculosis of the lungs, where the original lesion is a minute tubercle the process extends peripherally with the production of a large number of daughter tubercles which coalesce and necrosis takes place in the centre of the mass till perhaps ultimately, a cavity of about the size of a cricket ball may be formed. It is not uncommon to find complete spontaneous healing of the tubercle

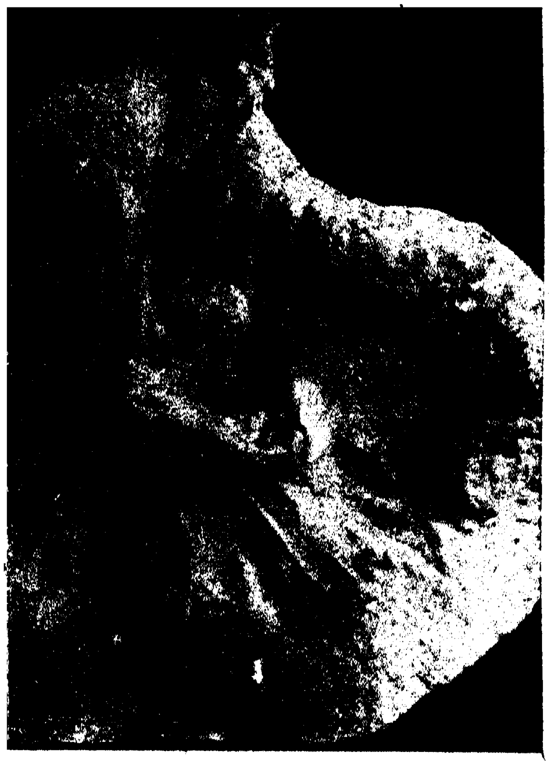

Fig. 165. Miliary tubercles of the mesentry.
(From Path. Museum Cal. Med. Col.)

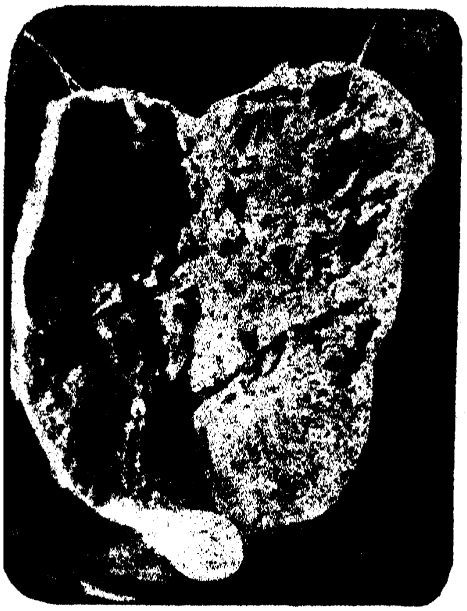

Fig. 166. Tuberculosis of the lung. (From Path. Museum Carmi. Medical College).

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* It will be convenient to call all lesions caused by the tubercle bacillus as *tuberculous* in contradistinction to similar tubercle-like bodies produced by other agencies which may be termed as *tubercular.*

even after caseation has taken place. This is very often seen in case of tuberculous peritonitis where on exploratory laparotomy it may be found to be studded with numerous tubercles which is found to present a smooth and normal appearance, if the abdomen is again opened after several months.

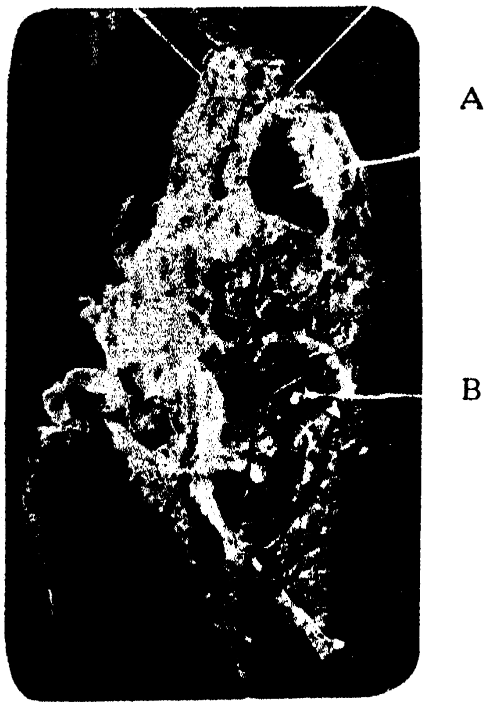

Fig. 167. Tuberculosis of the lung.
A—Cavity.
B—Calcified nodule.
(From Path. Museum Calcutta Medical College).

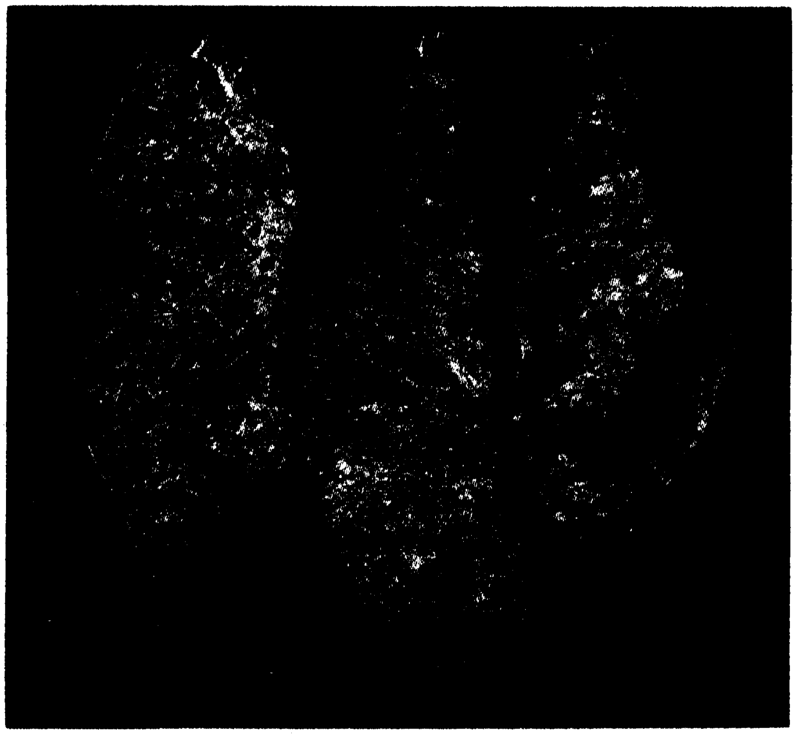

Fig. 168. Tuberculosis of the lung.
(From Path. Museum Carmichael Medical College).

tion. As the stage advances, secondary tubercles form surrounding the original one with involvement of the vascular supply of the central part resulting in necrosis. The central necrosis is not only due to the want of blood supply, but mainly due to the direct action of the toxin of the tubercle bacillus on the tissue elements. This toxin may also lead to thrombosis and necrosis of the capillary wall thus producing further avascularity.

Fresh tubercles appear surrounding the necrosed zone until all the tubercles coalesce forming one single mass with caseation at the centre. In the case of an acute process the extension is rapid and encapsulation rare; whereas in chronic conditions a large amount of fibrous tissue surrounds the focus and may often check the further progress. In very chronic conditions the encapsulation may be complete and no further tubercles form outside the capsule. Evidently this is due to the diminished virulence of the organisms. In such a condition calcareous particles accumulate in the necrosed mass which is either absorbed or calcified.

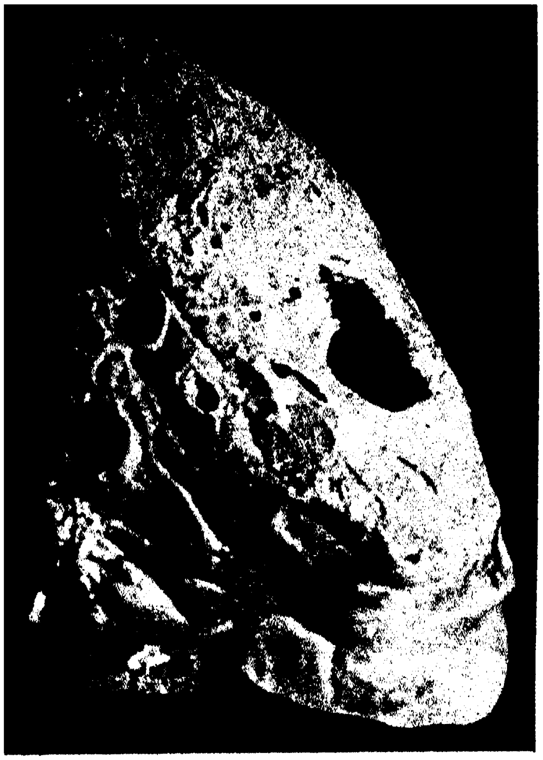

Fig. 169. Tuberculosis of the lung—Showing cavity surrounded by consolidation. (From Path. Museum Carmi. Med. Col.).

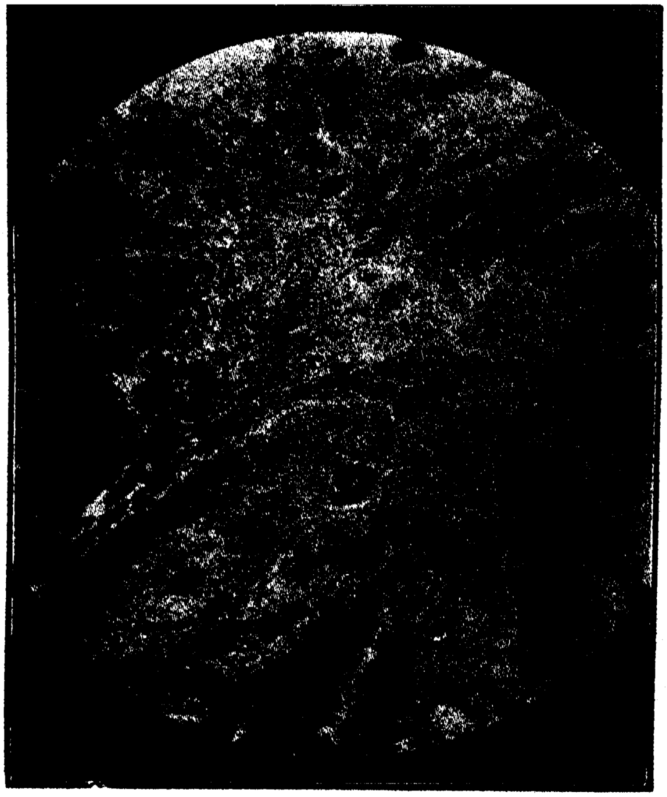

Fig. 170. Tuberculosis of lymphatic gland. Numerous giant cells with many deeply stained nuclei arranged along the periphery. Numerous small round cells.

In the lungs, in the early stages, the irritation produces congestion and formation of a large number of tubercles which by their coalition form areas of consolidation. These consolidated areas undergo caseation, and the caseous matter is subsequently expectorated with the sputum forming cavities.

Hence three stages of pulmonary tuberculosis are usually met with *viz.*, (1) congestion, (2) consolidation, (3) cavity formation.

The above process is true for all tuberculous conditions whether of glands, organs, bones or joints.

Histology.—The structure of a tubercle consists in the centre of a zone of necrosis which stains badly (if the necrosed mass has been already evacuated, a cavity is found). Surrounding the area of central caseation there is an area consisting mainly of endothelial cells and giant cells. The latter are characterised by the presence in them of a large number of nuclei arranged peripherally in a crescentic manner. This is the typical appearance of Langhan's giant cells. The nuclei may even form a complete circle. They may be arranged in a single layer or occasionally in 2 or 3 layers. The margins of giant cells are not regular, often showing branching processes. Sometimes neighbouring giant cells are united with one another by means of these processes. Surrounding the endothelial cells and giant cells there is a zone of small round cells which in their turn are surrounded by a zone of fibrous tissue.

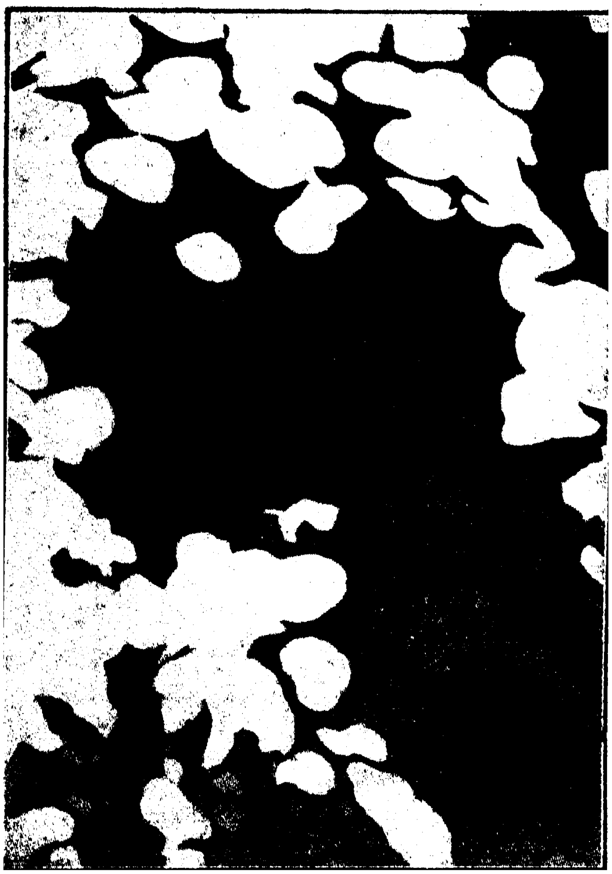

Fig. 171. Tuberculous consolidation of the lung, showing giant cells.

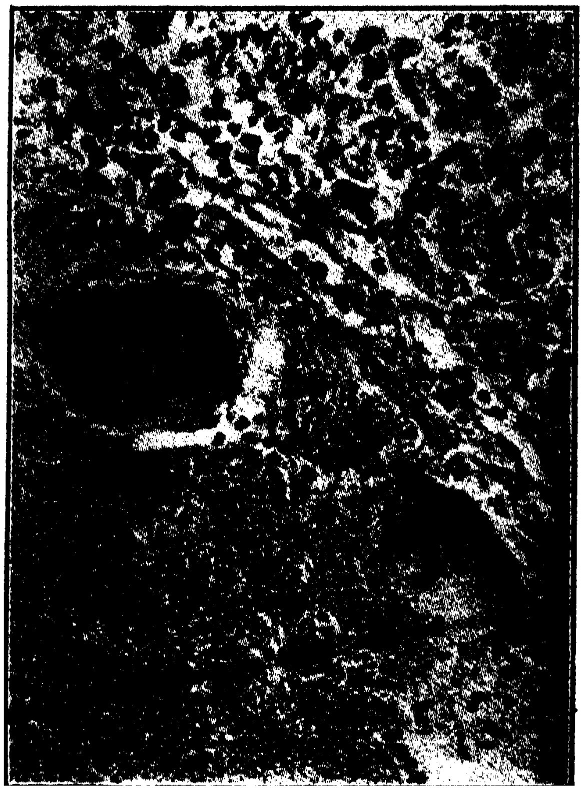

Fig. 172. Portion of the fig. 171, more magnified showing giant cells with round and endothelial cells above and caseation below.

All the three types of connective tissue fibres, *e.g.*, collagen, elastic and reticulum fibres, as met with in the lung are affected by tuberculosis. In tuberculosis, the collagen fibres are destroyed early but proliferate again when the resistance of the individual is raised and the virulence of the organisms become low. The elastic fibres are also lost early and do not proliferate early. The reticulum fibres, on the other hand, play a very important part and serve as an index of the defence reaction. The more the power of resistance, the more the development of the reticulum. The reticulum is seen to be coarser and thicker along with the appearance of the giant cells and caseation. The caseated area which, with the hæmatoxylin and eosin stain, appears homogeneous and structureless, appears full of reticular fibres. In gelatinous pneumonia, on the other hand, where the resistance is extremely low and virulence of the organisms very high, there is complete absence of any reaction of reticulum as well as other connective tissue fibres and also absence of any formation of giant cells and tubercles. There is only exudative changes taking place.

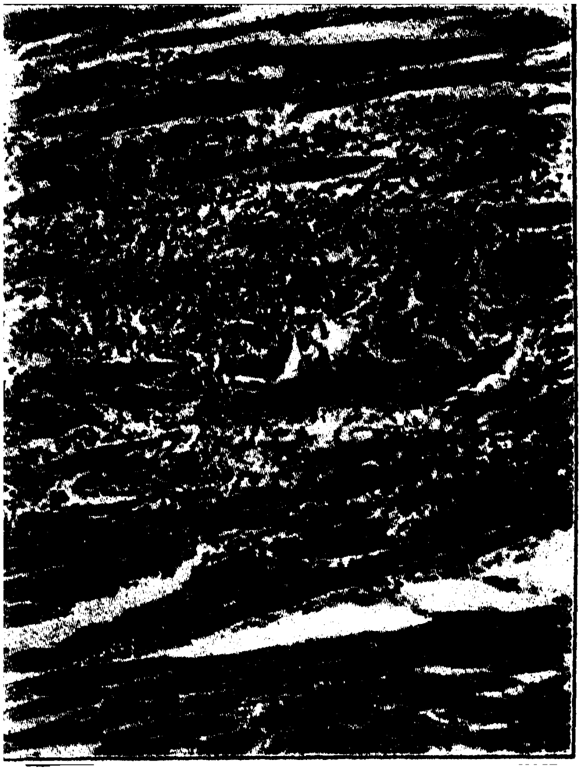

Fig. 173. Tuberculosis of the heart.

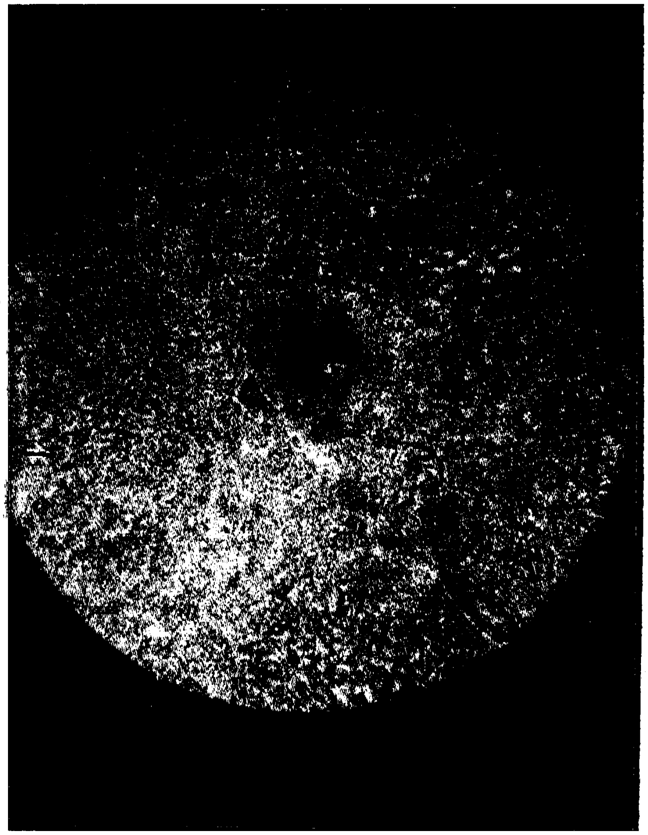

Fig. 174. Tuberculosis of Liver.

The course of histological events in the tubercles may be as follows: (1) During the first week, the epitheloid cells appear; (2)

at the end of the first week, the round cells make their appearance; (3) at the end of the second week, starts the caseation.

Termination.—Nutritional disturbance is the rule in all tuberculous lesions, hence, sooner or later caseation takes place. The cellular elements undergo cloudy swelling and fatty changes and form a cheesy mass. In case of chronic conditions it is encapsulated and in some instances may be calcified. Calcification does not mean that the organisms are dead. They remain dormant and may set up an inflammatory process at a later date if the soil becomes favourable.

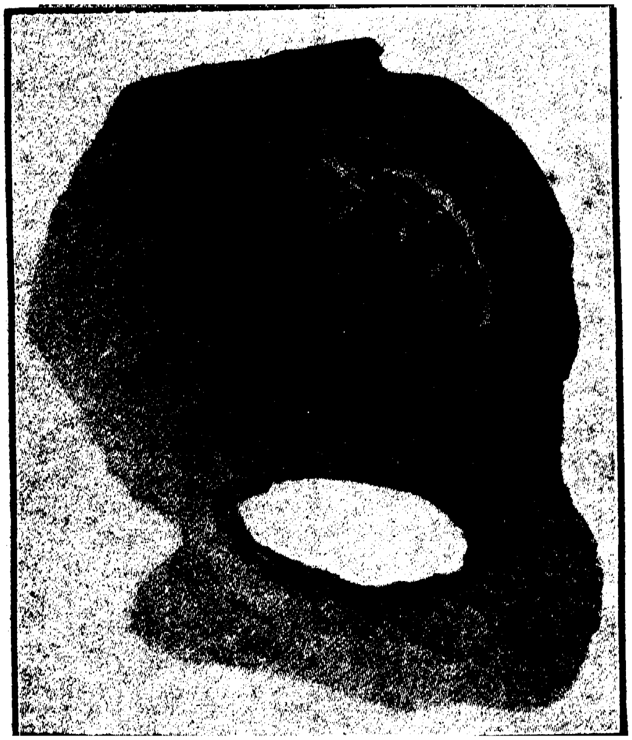

Fig. 175. Tuberculosis of the thoracic duct.

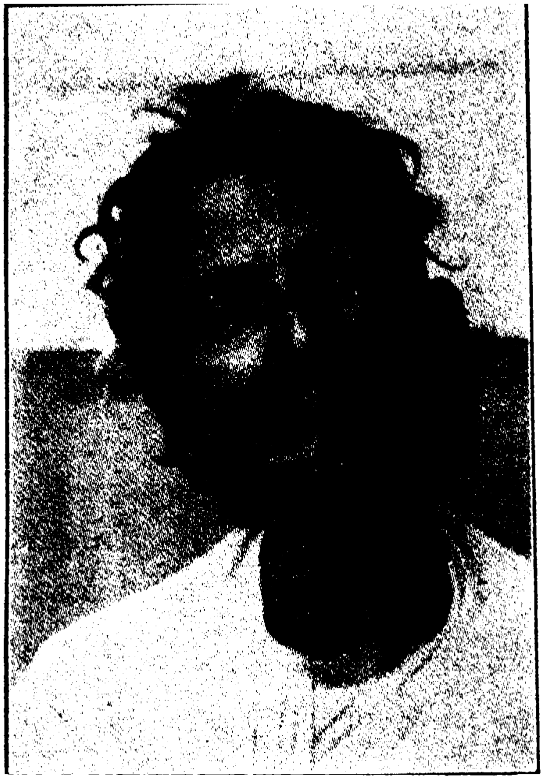

Fig. 176. Lupus. (From Dr. Nagendra Nath Das's Collection).

Tuberculosis in Animals.—Animals are also liable to be infected by B. tuberculosis. *Guineapigs and rabbits* are usually employed in the laboratory for the diagnosis of tuberculosis as they are very susceptible to the infection. Domestic animals particularly the *cattle* are often naturally infected. Tuberculous infection of cattle is extremely common in temperate climates and hence is of extreme importance. 30 to 50 per cent. of the cattle in England are infected The important point is that the infected cattle show no symptoms of the disease unless in an advanced conditon. In India 1 to 1.5 per cent. of the cattle are infected.

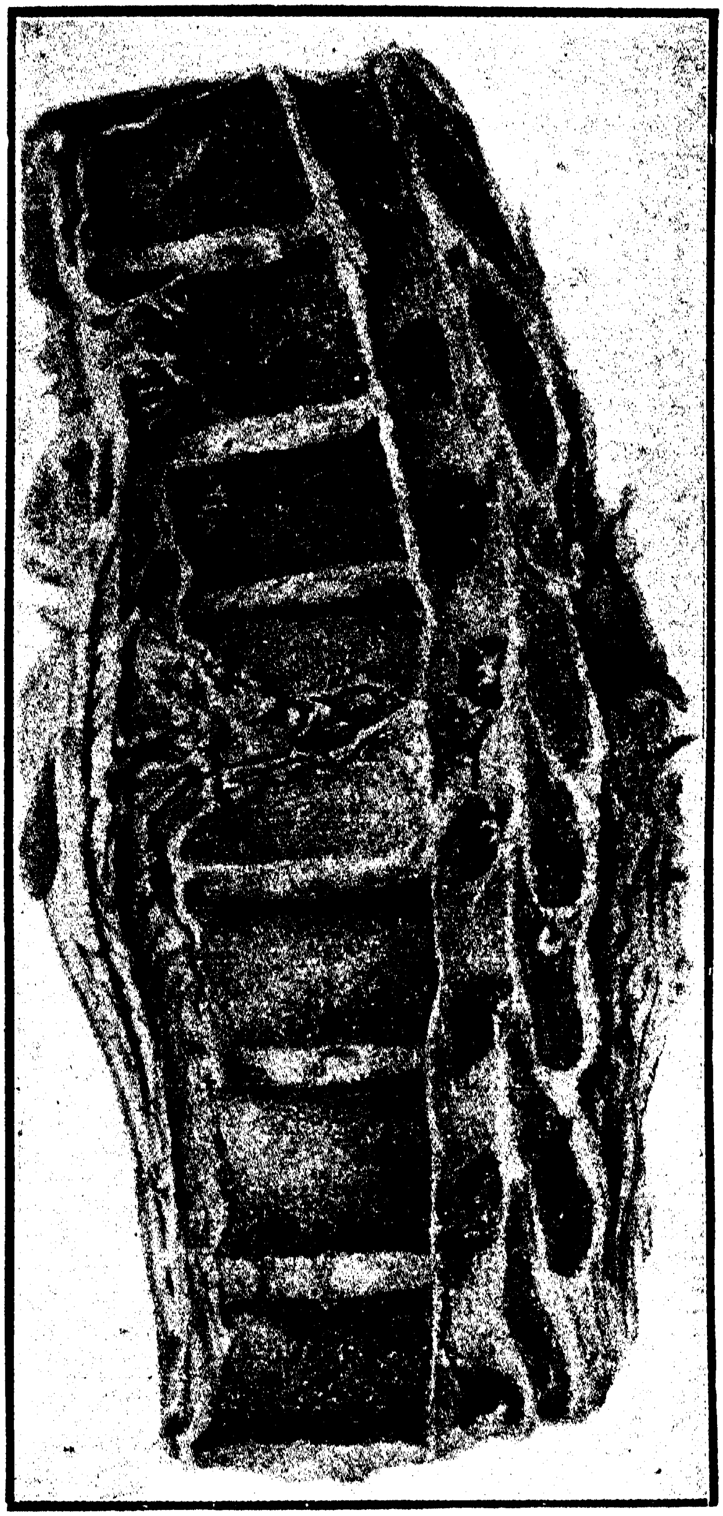

Fig. 177. Tuberculosis of the Spine. (From Path. Museum Cal. Med. Col.)

This is the reason why in Europe tuberculous disease of the glands, bones and joints are so common, especially amongst children. In addition to cattle, pigs, monkeys, goats, cats, horses and birds are also found to be infected naturally. The view that goats are immune to tuberculosis *has been found to be erroneous.* Bovine T. bacillus is more virulent to cattle than the bacillus of the human type. In rabbits, the human type produces only local lesions, whereas the bovine type invariably kills them.

Experimental tuberculosis in laboratory animals, —Intraperitoneal or subcutaneous inoculation into guineapig will show—(1) Early inflammatory changes for 2 to 3 days. (2) Mononucleated endothelial cellular zone round the organisms—in 3 to 5 days. (3) Lymphocytic infiltration surrounds the tubercle in about 6 to 10 days. (7) Necrosis of the centre (caseation) and formation of giant cells takes place in about 15 days. (5) Enlargement of the nearest lymphatic gland in case of subcutaneous injection in about a week. (6) Dissemination of tubercles in the lungs and other organs in about a month.

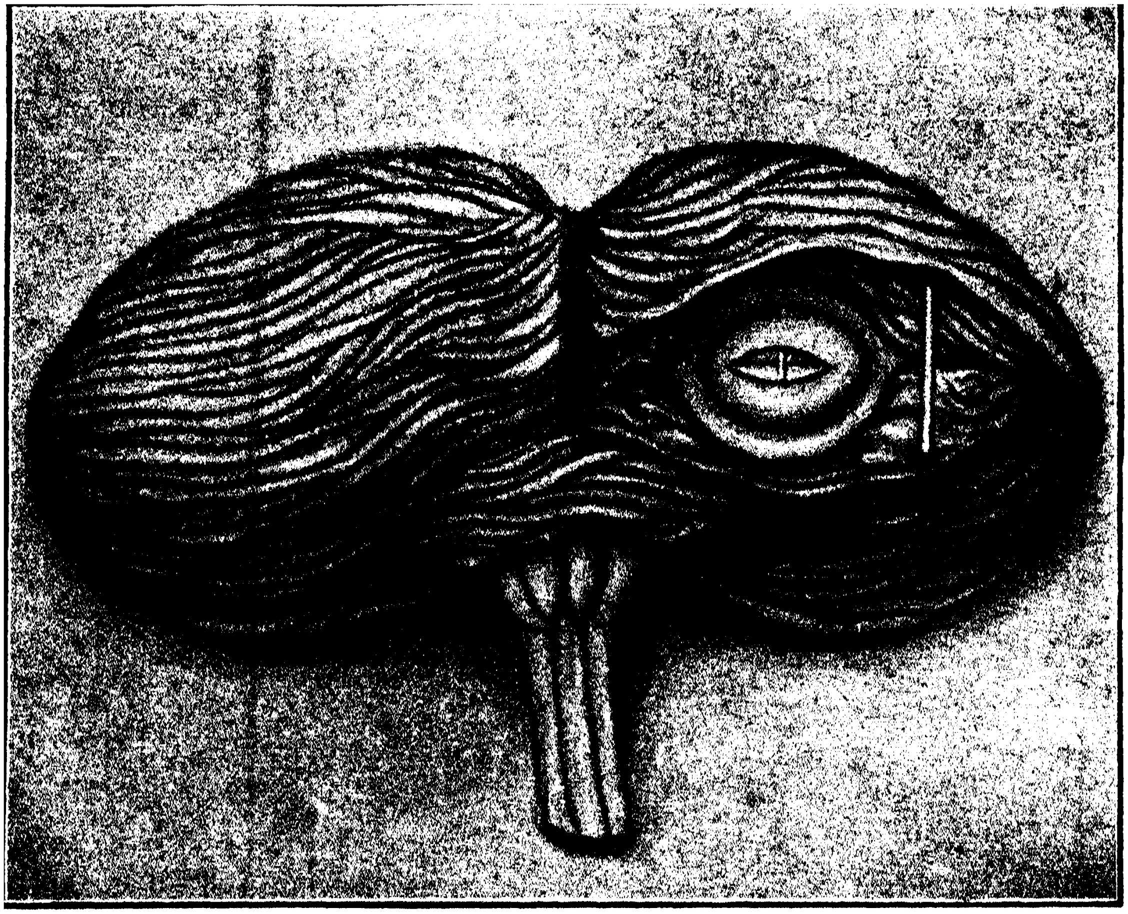

Fig. 178. Tuberculous tumour of the cerebellum.
(From Dr. Bidhan Chandra Roy's Collection).

Koch phenomenon.—The behaviour of the animal with the second dose of tubercle bacillus subcutaneously is remarkable and is known as the Koch phenomenon. Unlike the lesions of the first injection, a second dose given one to two weeks after the first injection will produce in a few days an indurated area, which soon sloughs but the ulcer heals quickly and permanently with no involvement of the regional lymph nodes. This phenomenon is due to the production of immunity by the first dose. Calmette is of opinion that the breaking down of the tissue with formation of cavity is to be regarded as the more or less successful attempt of the tissues to expell the bacilli.

SYPHILIS

Origin.—It is generally believed that this disease was first introduced into the civilized world by the sailors of Columbus on their return to Europe after the discovery of the New World in 1493. It is said that the inhabitants of Hayti suffered from the disease from time immemorial and infected the sailors of Columbus, who again in their turn spread the infection through Italy to France and other countries.

Etiology.—It is a specific infectious disease caused by a spiral organism known as the Treponema pallidum.

Infection.—The tendency of the organism is to spread rapidly so as to produce a generalised condition with or without local manifestations. The infection primarily takes place through some abraded cutaneous or mucous surface. This is usually transmitted by coitus but may also take place through other contacts, *e.g.*, kissing, use of infected razor, utensils, etc. Surgeons infect themselves through fingers during operation of a surgical lesion and the gynæcologists during a vaginal examination.

Course.—For the sake of convenience the course of the disease is divided into several stages. They are as follows:—

(1) *Incubation Period.*—This varies greatly in different individuals. On the average, the period is about 3 weeks but may vary from 10 to 40 days.

(2) *Stage of Primary Lesion.*—The primary lesion appears as a characteristic local lesion at the seat of inoculation which ulcerates with the formation of what is known as a chancre. The infection also involves nearest lymph glands and leads to the formation of adenitis.

(1) The Chancre.—Also known as *Hard Chancre, the Hunterian Chancre.*

Site.—It is most frequent in the genital regions; in the male—upon the glans penis, outer or inner surface of the prepuce, in the meatus urinarius, the urethra, scrotum and groin; in the female—upon the labia majora, clitoris, vagina, cervix uteri and the skin of the groin. Apart from the genital regions, chancres are often found on extragenital surfaces. They are as follows: regions near the anus, lips, mouth, cheek, tongue, palate, tonsil, nipple, finger or any other surface exposed to the infection.

Morbid Anatomy.—The chancre begins as a superficial small papule rapidly changing to a vesicle. It is surrounded by an area of induration and often the surface epithelium becomes necrotic with formation of a small shallow ulcer. The ulcer extends in every direction both in depth and in the sides. With the extension of ulceration the surrounding induration also extends giving a feel of hardness at the margin of the ulcer—hence the name *hard* chancre, so as to differentiate from *soft* chancre which is a quite distinct affection and is caused by a different organism, the bacillus of Ducrey. The chancre is usually a single one but may rarely be multiple. It is invariably present at the seat of inoculation alone and nowhere else. It is found in all cases of syphilis expect the congenital form. It heals either spontaneously or under treatment but the induration often remains permanently.

Histology.—The most prominent feature is the presence of—(*a*) An abundance of round cell infiltration at the base of the ulcer, and specially surrounding the blood vessels. These cells are mainly the lymphocytes, plasma cells, large mononuclears and rarely polymorphonuclear leucocytes. The polymorphonuclear leucocytes are present on the superficial aspect of the ulcer. (*b*) Large number of endothelial cells and wandering cells. (*c*) Blood vessels—fairly numerous—the inner layer of which are greatly thickened. (*d*) Proliferation of fibroblasts surrounding the ulcreated area, which along with the other cellular elements give the induration and hardness of the lesion.

Adenitis.—The neighbouring lymph glands are enlarged and become hard. As the genital region is the frequent seat of the chancre, the inguinal glands are commonly affected. The glands show little tendency to adhere to the neighbouring tissues and hence feel shotty; they are moveable under the skin, painless and never suppurate.

Histology.—The lymph channels are packed up with round cells and wandering cells surrounded by a large accumulation of fibroblasts.

(3) *Stage of secondary incubation.*—After the healing of the chancre there remains no other evidence of the disease except perhaps the shotty glands. This stage of secondary incubation lasts for about six to ten weeks.

(4) *Stage of secondary symptoms.*—This stage lasts for one to three years and is characterised by a large and extraordinary variety of skin and mucous lesions, of more or less generalised affection of lymphatic glands, fever, affection of joints, malaise, anæmia and other constitutional disturbances.

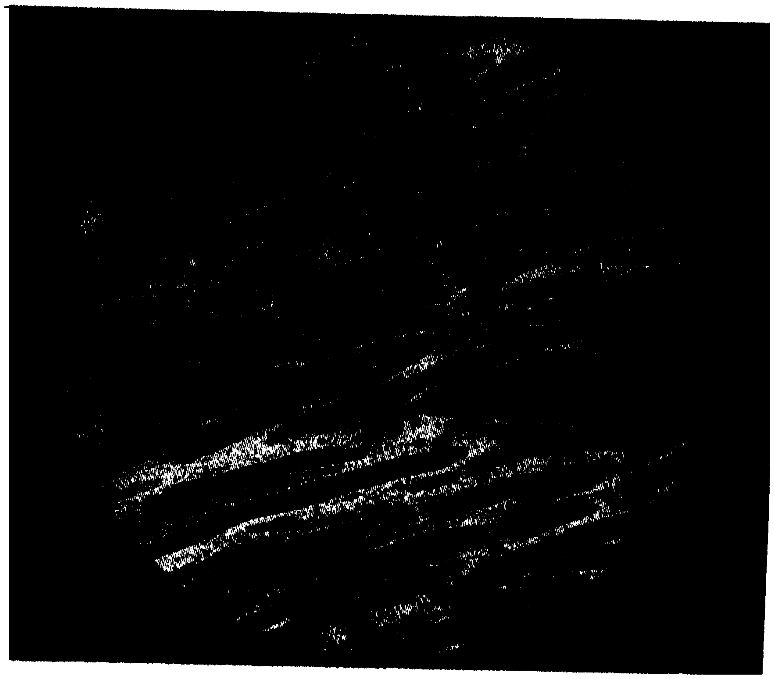

Fig. 179. Gumma of muscle showing large number of round cells.

(i) **Skin Lesions.**—These are both polymorphous and pleomorphous. They have a peculiar symmetrical distribution and are mainly found on the flexor surfaces.

There is no space here for even a brief description of the numerous secondary lesions caused by syphilis. They are macular in the early stages and papular, pustular, scanty and pigmented in later stages of the disease.

(ii) **Mucous Lesions.**—They are also extraordinarily variable. Sore throat is a very common condition. Mucous patches occur in the mouth, throat, larynx, vagina and other places. They are pale or reddish lesions with raised and superficially ulcerated surfaces.

(iii) **Mucocutaneous Lesions.**—Condyloma, they are usually found on the moist surfaces.

Histology.—The lesions in the secondary stage show widening of the blood vessels with perivascular infiltration. The superficial epithelium shows proliferation.

(5) *Stage of Tertiary Incubation.*—This stage lasts for a period of several years and may last even for the rest of the life of the individual.

(6) *Stage of Tertiary Symptoms.*—This stage lasts for an indefinite period. The characteristic lesion in this stage is the *Gumma* which is composed of granulation tissue with degenerative conditions. The degeneration is more or less elastic and is of gummy consistency, hence the term gumma.

The **Gumma** may be single or multiple. The size varies from a pinpoint to the size of a cricket ball. No part of the body is exempted from its occurrence.

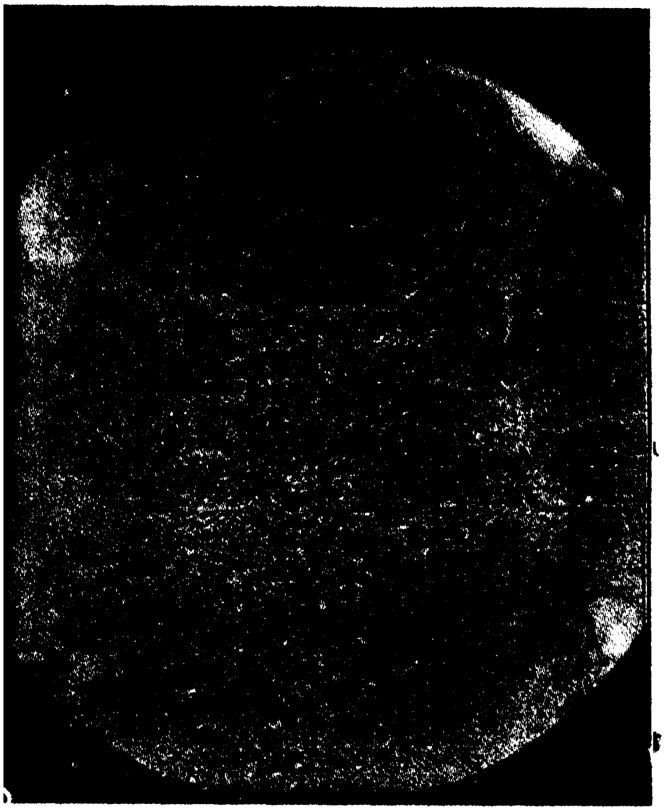

Fig. 180. Gumma showing endarteritis.

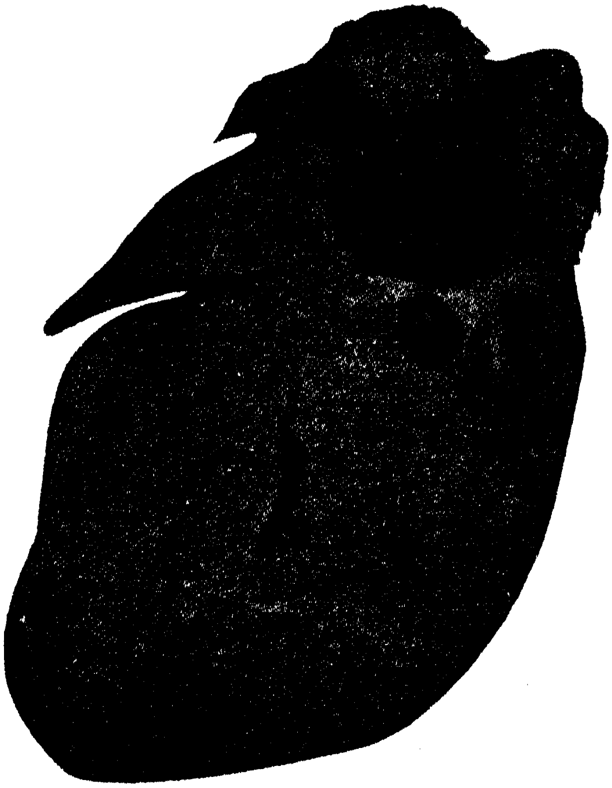

Fig. 181. Gumma of the liver. (From Path. Museum Carmi. Med. Col.).

Morbid Histology.—This consists of a necrotic centre with or without giant cells and is surrounded by a large number of round cells. In the early stages proliferation of endothelial cells takes place in the centre.

Blood vessels show characteristic changes. The wall is greatly thickened and occasionally found to have been totally obliterated—the so-called endarteritis.

Syphilis causes endarteritis, mesarteritis and also periarteritis.

The vasa vasorum is also greatly thickened and often surrounded by a dense accumulation of round cells. The media of big arteries shows numerous breaches in the elastic and also in the fibrous tissues due to the affections of vasa vasorum and also shows areas of round celled infiltration.

Subsequent Changes.—The usual tendency of the gumma is to evacuate itself. This is possible where it is situated superficially near the cutaneous surface or in the neighbourhood of any cavity or channel. In case of liver and other internal organs where there is no similar opportunity it is left to be absorbed in course of time, leaving a dense amount of fibrous tissue with the formation of a thick scar, which is absorbed very slowly or imperfectly, or deposition of calcareous particles takes place, resulting in calcification of the necrotic substance.

Other lesions in this stage are—Cutaneous lesions—rupia, etc.; Bone lesions—periostitis, ostitis, osteomyelitis, osteoperiostitis; Muscular lesions—myositis, contractures,etc.; Nervous lesions—neuritis, myelitis, meningitis, cerebrospinal syphilis, etc.; Vascular lesions—arteriosclerosis, aneurism, thrombosis, etc.

(7) *Meta-syphilitic Affections.*—These are tabes dorsalis and general paralysis of the insane (G.P.I.).

(8) *Congenital syphilis.*—This is due to the infection transmitted to the fœtus.

(*a*) Hereditary, *i.e.*, through the ovum or the spermatozoon or both. (*b*) In the course of its development from postconceptional syphilis of the mother. It is said that postconceptional syphilis may be transmitted to the fœtus *in utero* up to the seventh month of pregnancy.

From the above it is evident that the term congenital syphilis is more appropriate than that of hereditary syphilis.

Mode of Infection and Manifestations.—This has been well summarised by McFarland as follows:—

" The hereditary form of the disease lacks the primary stage. Instead of micro-organisms reaching the child through an external focus where it first forms a chancre, and then invades the lymphatics, they enter from the germinal cells, grow and multiply simultaneously with the developing embryo, or in case of later infection of the mother, having produced its early manifestations in the body and invaded her blood, pass through the placenta and enter the circulation of the offspring.

" Prenatal infection with syphilis is a very serious obstacle to the proper development of the embryo. Exactly how it will interfere with its progress will depend upon the time at which the infection of the embryo occurs and the activity of the disease in the parents. Thus, if the parents suffer from active and malignant secondary

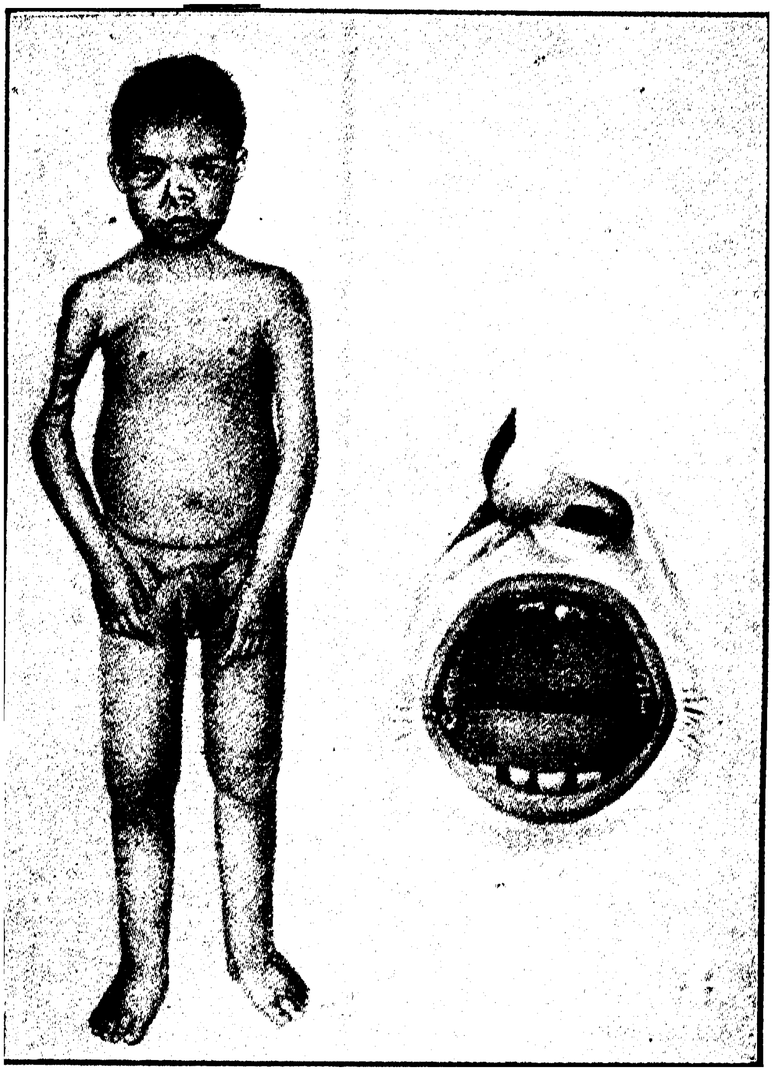

Fig. 182. A Heredo-Syphilitic child with Hutchinson teeth, perforation of the palate, epiphysitis nephritis with general anasarca and radiating scars at the angle of the mouth. (Circum-Oral Syphilide.) (*From " Syphilis "—K. K. Chatterjee.*)

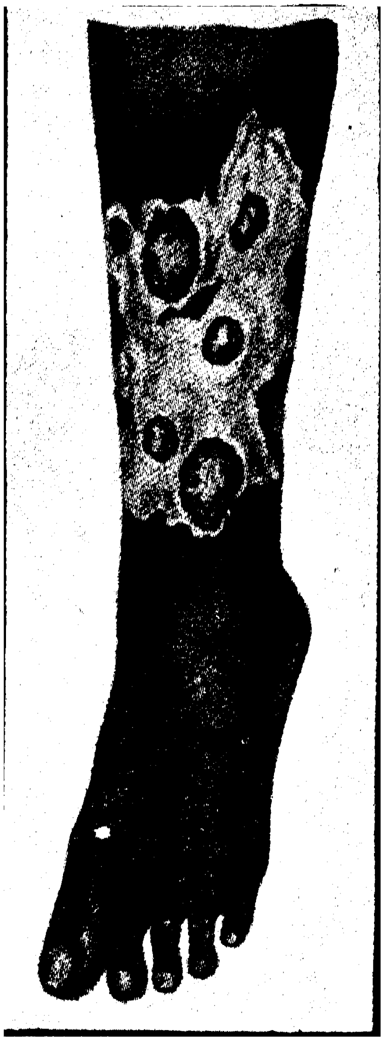

Fig. 183. Ulcerative Gummatous Syphilide of the Leg. There are typical gumatous ulcers with wash-leather surface and cicatrices. (*From " Syphilis "—K. K. Chatterji.*)

syphilis at the time of procreation, the disease in the embryo is usually so active as to cause its death and lead to abortion. The aborted embryo is apt to show exaggerated lesions, such as palmar pemphigus and disease of the internal organs. If the activity of the disease be more mild, the fœtus dies later and is born dead and macerated. It is said that at least one-third of syphilitic children are born dead.

"If the virus is still less virulent, the children may be born alive with evidences of the disease, when they usually die shortly, or without evidences of its presence, to develop the symptoms and fall into an unhealthy condition shortly after.

"Of these healthy appearing children it is said that about 25 per cent. die in the first six months. Even if they survive longer, the dangers are overwhelmingly against the subsequent attainment of health and freedom from deformity.

"The newly born child of syphilitic parents usually shows no symptoms of the disease for from one to three weeks. About 30 per cent., show manifestations within a month. 66 per cent. of the remainder within three months.

"The appearance of the syphilitic child, whether the syphilis be hereditary or accidentally acquired through contact with mucous patches in the maternal tissues during delivery is about the same, the effect of the disease being a marked impairment of nutrition and growth.

"The child appears small, withered, and weazened. Its features are senile or simian. It has a hoarse cry, and suffers catarrh of the nasal mucous membrane, which causes the characteristic 'snuffles.' Cutaneous eruptions are frequent, and changes in the bones and joints are partly characteristic.

"If the child lives, its life is apt to be a continued succession of morbid processes, pemphigus, coryza, cirrhosis of the liver, osteochondritis, dactylitis, interstitial keratitis, etc.

"All its physiologic processes are disturbed and its structure morbidly modified. Thus, the teeth of the first dentition are irregular in development, opaque, chalky, deficient in enamel, with soft friable dentin, inequality in size, and proneness to decay. The permanent teeth are characterized by a peculiarity of the upper central incisors, known as "Hutchinson's teeth" the fang of each tooth being short, thick and deeply notched at the edge.

" As the child grows, various cellular infiltrations, superficial and deep ulcerations, make their appearance, with corresponding cicatrizations and more or less marked disfigurement and deformity."

LEPROSY

Leprosy is a specific infectious disease caused by B. lepræ of Hansen and characterised by the formation of granulomatous lesions in the skin, mucous membranes and nerve sheaths.

History.—It is existing from ancient times all over the world and was generally confounded in ancient literature with syphilis and psoriasis. The causative organism was discovered by Hansen in 1871 and the clinical study of the disease was first done by Danielson in 1847.

Methods of infection.—This has not yet been known definitely. The organisms are present in every leprous tissue and it is supposed that they leave the person through the nasal secretions, discharges from the ulcerated surfaces of the skin and the nodules and also with the sputum, fæces, urethral and vaginal discharges, tears, saliva, milk, etc. Of these the nasal secretion is the most important one. Various experiments have been made to infect lower animals with leprotic material and the so-called culture of the organisms, but so far not with any success. Partial success has been reported with the chimpanzee.

As regards experiments on human beings the only one attempted was that on a criminal in the Sandwich Islands who developed, four weeks after inoculation in the arm, a neuritis of the ulnar and the median 'nerves. Two years and a half later he developed all the signs of leprosy and died of it six years after the inoculation. The value of this important experiment, however, decreased to a considerable extent from the fact that the person came from a leprous country and from a leprous family.

There is practically no doubt that it is transmitted from one person to the other. Father Damien, a missionary from Belgium, who lived among lepers of the Leper Asylum of the Sandwich Islands in 1873 showed evidences of the disease in 1882 and subsequently died of it in 1889. There are numerous other instances of men who acquired the disease in a leprous country, came back to his nonleprous home and subsequently transmitted the disease to the members of his family, who lived in close personal contact with him

Isolation, again, controls the spread of the disease to a great extent indicating that it is more or less transmitted directly. Isolation of apparently healthy children from leprous mothers often prevent the spread of infection. Further, it is also doubtful whether sexual intercourse transmits the disease from the husband to the wife. Nor there is any proof that the infection is transmitted through the spermatozoon or the ovum.

The evidence in favour of the transmission of the disease from one person to the other are—(1) Direct contact with the lepers, and (2) Transmission by an insect, *e.g.*, bites of mosquito, pediculi, sarcoptes scabiei and also by, house fly, etc.

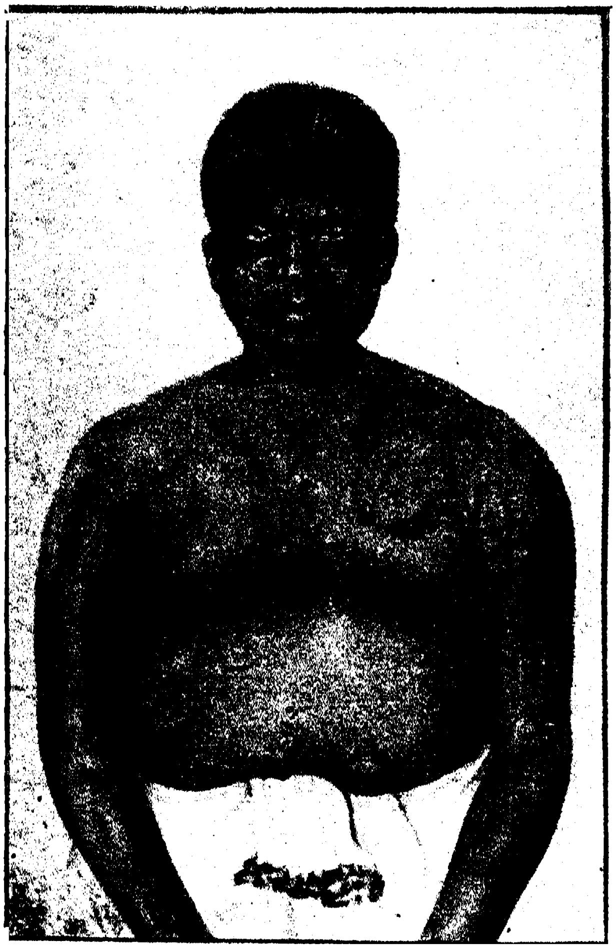

Fig. 184. Leprosy (anæsthetic form). (From Dr. Bidhan Chandra Roy's Collection).

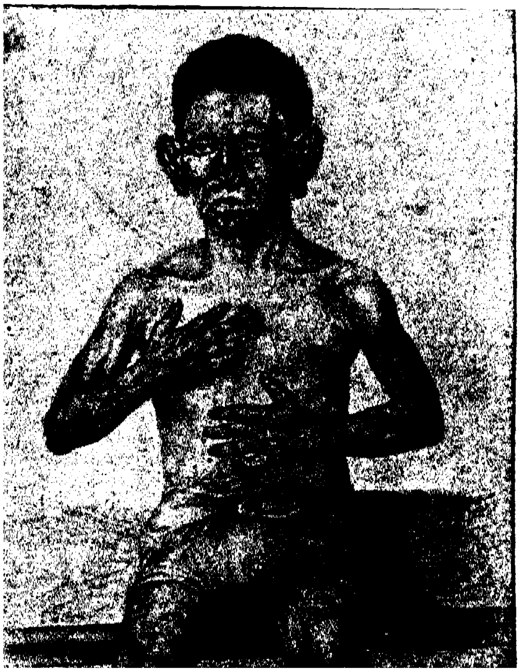

Fig. 185. Leprosy (Nodular form). (From Path. Museum, Cal. Med. Col.).

Route of Infection.—It is supposed that the infection takes place either through the skin or mucous membrane of the nose and respiratory tract. On entering the tissues the organisms enter the lymph channels where they multiply, forming the so-called lepra cells, and subsequently disseminate throughout the body either through the blood or lymph channels.

Morbid Anatomy.—This is usually described under two clinical types—(1) nodular type, (2) maculo-anæsthetic type.

(1) *Nodular Type.*—This is due to the infiltration under the skin with formation of nodules distributed all over the body being most frequent on the face and the extremities. In the face the nodules appear, all over the forehead, cheeks, ear, lips, chin, sometimes in fairly large size with great disfigurement of the appearance of the face by obliterating the natural facial lines and developing new lines between neighbouring nodules. The face looks that of a lion and hence the term "leontiasis." Affection of the eyes with similar infiltration of conjunctiva, sclera, iris, eyelids, nose, tongue, mouth, pharynx, larynx, etc., are fairly common.

(2) *Maculo-anæsthetic Type.*—In this type the sheath of the peripheral nerves and especially the ulnar, the median, and the posterior tibial nerve fibres are involved. The infiltration leads to irritation of the nerve fibres followed by degeneration of the nerve.

The earlier manifestations are therefore due to the irritation of the nerves, *e.g.*, pain, with other sensory disturbances, *e.g.*, numbness, burning etc., and also motor disturbances, *e.g.*, twitchings and spasm of the muscles supplied by the nerve. The skin supplied by the affected nerve shows macular eruptions followed by loss of hair of the part which subsequently becomes anæsthetic.

The nerve appears thickened due to the infiltration and the muscles are often found paralysed and wasted. Trophic lesions in the form of perforating ulcers, etc., are also present.

Histology.—The section of a leprous nodule of the skin shows more or less intact superficial epithelium with no interpapillary processes. In the deeper layers it presents the appearance of a granuloma with large number of round cells, plasma cells, endothelial cells, lepra cells, fibroblasts with a fair amount of fibrous tissue supporting the blood vessels which also show perivascular infiltration.

Of these cellular elements the *lepra cells* deserve special mention. These are big cells containing large number of lepra bacilli arranged in bundles. (See plate I.).

There are different views as regards the origin of these cells. Some believe that they are endothelial cells which have swallowed large numbers of the organisms, while others are of opinion that what we call cells, are strictly speaking lymphatic channels packed up with lepra bacilli.

GLANDERS

Glanders is a specific infectious disease caused by bacillus mallei. It is mainly an infection of the horse. It also occurs in goats. It affects man chiefly as a secondary infection of some wound of the skin and other parts of the body from the discharges of an infected animal. Thus, persons who work mainly in close contact with horses, are more liable to the disease.

Morbid Anatomy.—The disease occurs in two forms in the horse: (1) *Glanders proper.*—In which the nasal mucous membrane is commonly affected extending deep into the septum and neighbouring parts with the formation of nodules which ultimately soften and break down with the formation of ulcers. The cervical lymph glands are nearly always affected extending to those in the mediastinum and also to the lungs, liver, spleen, etc. (2) *Farcy.*—This is the cutaneous affection of glanders, usually taking place through some abrasions. Secondary affection of the glands are frequent.

In man it occurs in two forms: (1) *Acute Form.*—In this the seat of inoculation, shows extensive inflammatory conditions with marked swelling and redness. It rapidly spreads to the whole body with the formation of a large number of eruptions, at first papular, rapidly changing into pustular form with extensive suppurative areas both on the surface as well as in the internal organs and ends fatally. (2) *Chronic Form.*—This is characterised by the formation of granulomatous nodules which break down and form irregular ulcers with foul discharge.

Histology.—The nodules and other lesions show changes common to other granulomas.

ACTINOMYCOSIS

Actinomycosis is a specific infectious disease caused by Actinomyces bovis and characterised by formation of nodules of granulation tissue. The organism belongs to the genus Streptothrix and was first observed in the lesions of cattle by Bollinger in 1876. It has a very wide geographical distribution. It is a common disease of the cattle occasionally infecting man. (See Figs. 22 and 23).

Morbid Anatomy.—The lesions are of chronic inflammatory type with tumour-like swelling usually of the jaws, terminating in suppuration in which the sulphur-grain bodies are discharged. In internal organs, *e.g.*, the liver it generally forms multiple lesions with honeycomb appearances due to numerous abscess cavities. In the late stages some of the abscess cavities may join together to form large areas. The process is chronic but progressive, muscles, bones and all the other tissues are disorganised to a considerable extent.

Histology.—The lesion consists of granulation tissue which shows signs of necrosis of the cells in the centre and accumulation of large numbers of leucocytes. It is generally a very slow process. The central portion is necrosed while fibrous tissue is formed in the outer zone. In the necrotic mass the fungus exists as sulphur-grain bodies and are discharged with the necrotic material. Giant cells are rarely found.

MYCETOMA

This is also known as **Madura Foot** due to the fact that this disease was first observed by Colebrook of Madura in 1846. The disease is widely distributed in India, Africa, Europe and America.

Varieties.—There are three forms of mycetoma—black, white and red according to the appearance of the granules. Of these the black variety is the commonest and belongs to the group of fungus known as hyphomycetes whereas the other two forms belong to the genus streptothrix.

Morbid Anatomy.—The disease generally commences in the foot usually with a previous history of a cut or injury. It rarely affects the hand and more rarely the leg, knee and other parts of the body. The earliest lesion shows itself in the form of a small hard swelling with a small bleb on the surface. The bleb bursts, discharging a little purulent material along with the fungus granules. The surrounding tissue is indurated and similar nodules appear in the adjacent parts with openings in them. Sooner or later the whole foot is involved. It swells up enormously with numerous blebs and sinuses which run deeply into the tissues involving bone and other structures. The patient can walk on such a deformed foot and the affection is not very painful. It takes a very chronic course and shows no tendency to heal.

Histology.—The microscopical appearance is that of a granuloma with extensive degeneration of the tissues even of the bones, The walls of the necrotic area containing the mycotic grains show the formation of young connective tissue with large numbers of round cells, plasma cells, endothelial cells and a few giant cells. (*See* Figures 24, 25, 26, and 27).

LYMPHOGRANULOMA

(*Hodgkin's Disease*)

It is just one hundred years since Hodgkin (1832) reported the description of seven cases with enlargement of lymphatic glands. As Hodgkin and his subsequent workers all worked only with the macroscopical appearance of the lesions with no histological differentiation, naturally, various affections have been grouped under the term "Hodgkin's disease," notably tuberculosis and lymphosarcoma.

Etiology.—The etiology of the disease is still obscure. All the controversies, at the present day have been centred on one of the following four factors, (1) that it is a form of specific infective granuloma, (2) that it is an atypical form of tuberculosis, (3) that it is a neoplasm, and (4) that it is a transition form between a granuloma and a tumour.

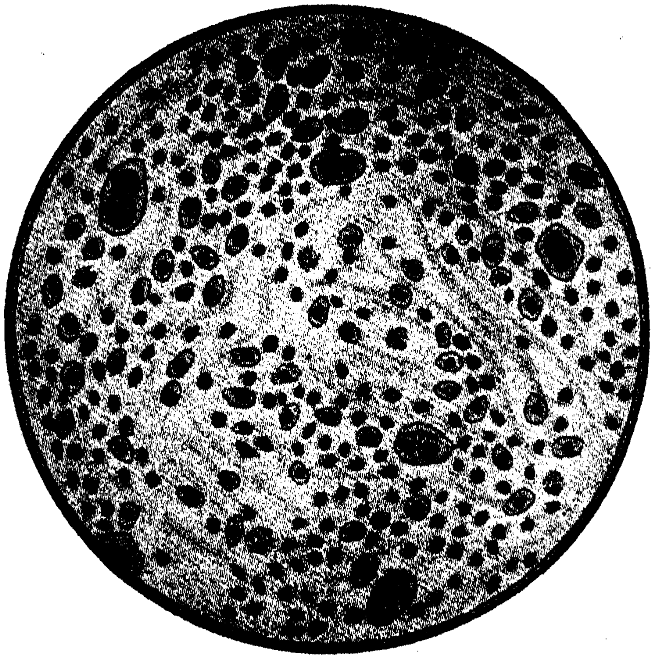

Fig. 186. Lymphogranuloma. (Hodgkin's disease).

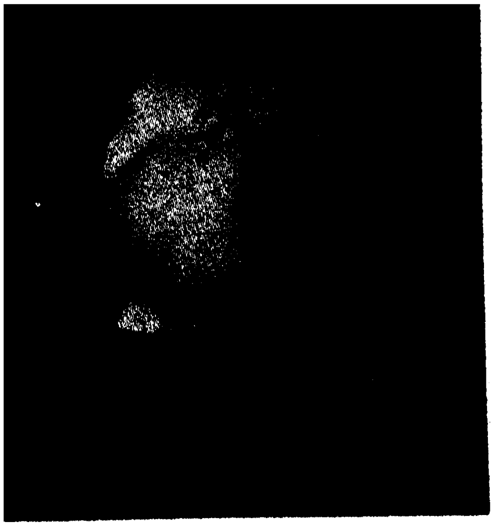

Fig. 187. Hodgkin's disease. (From Dr. P. K. Mitra)

1. *That it is a granuloma.*—Various workers have isolated pyogenic cocci and other organisms which could not be identified, from the blood of the patient suffering from Hodgkin's disease as

well as from the glands removed both before and after death. Others again isolated spirochaetes, a Gram positive pleomorphic diphtheroid bacillus in almost every case examined by them. They have even produced successful inoculation to laboratory animals. On the other hand, there are another host of workers who by careful repetition of the works done by the previous workers could not demonstrate any organism in the tissues. The general finding of most of the workers is that the tissue of Hodgkin's disease is sterile. Although no definite organism could be found, still from the typical histological characters of granuloma which is characteristic in Hodgkin's disease it is generally believed that this affection is a granuloma.

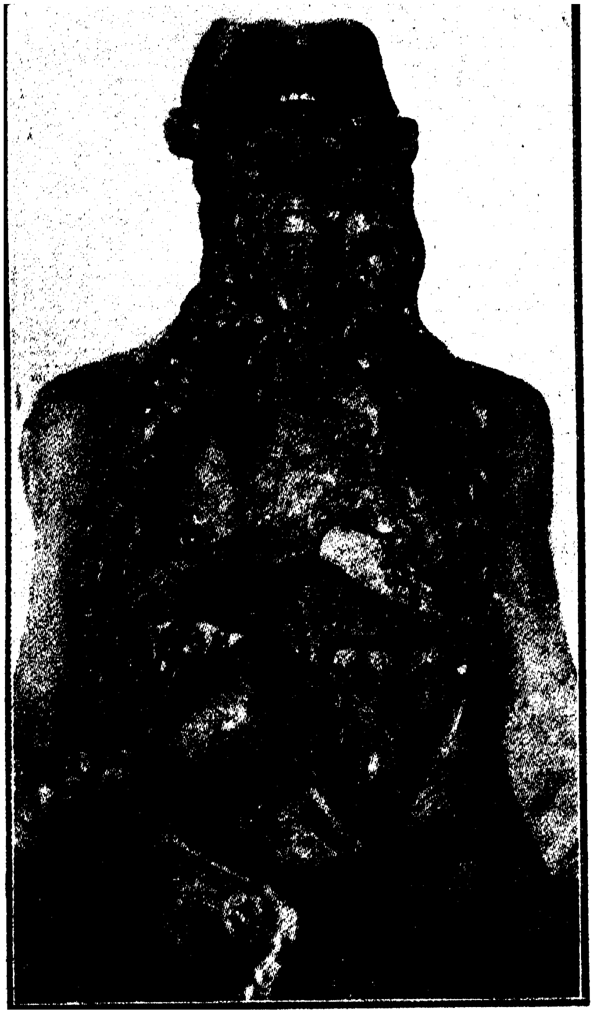

Fig. 188. Hodgkin's disease.
(From Path. Mus. Carmi. Med. Col.)

2. *That it is an atypical form of tuberculosis.*—It seems that three schools have developed regarding the question of the association

of tuberculosis and Hodgkin's disease. One school believes that Hodgkin's disease is nothing but tuberculosis, while others advocate the opposite theory and are of opinion that Hodgkin's disease has nothing to do with tuberculosis. The third school believes that occurrence of tuberculosis is either accidental association with Hodgkin's disease or that tuberculosis develops as a separate affection in a Hodgkin gland. There is a great deal of controversy between these three groups. The general opinion is that it has nothing to do with tuberculosis.

3. *That it is a neoplasm.*—The advocates of this theory regard this as a form of lymphoblastoma, and group this under the category of lymphosarcoma. Medler believes that the giant cells belong to the group of megakaryocytes. From this he believes that Hodgkin's disease is a malignancy of the bone marrow and uses the term megakaryoblastoma for it.

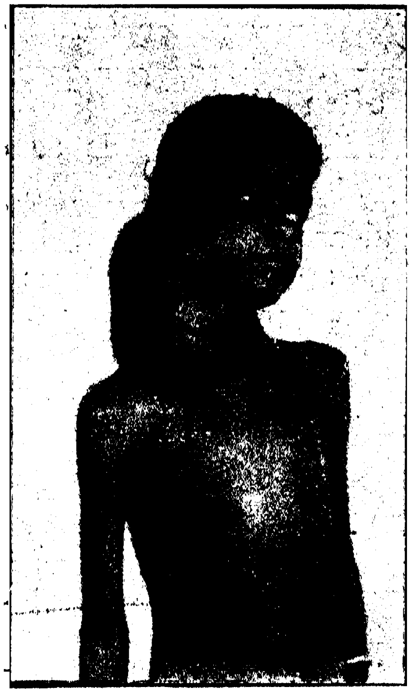

Fig. 189. Hodgkin's disease From Carmichael Medical College Hospital).

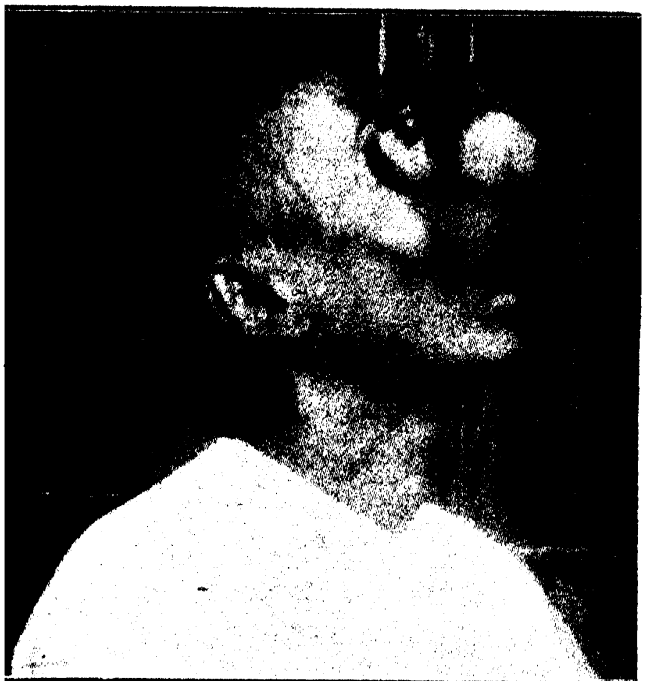

Fig. 190. Rhinoscleroma. (From Dr. P. K. Mitra).

4. *That it is a transition form between tumour and a granuloma.*—Like mycoides fungoides Hodgkin's disease has been regarded to be a condition holding a position between tumour and infectious granuloma. This theory has very few advocates.

Morbid Anatomy.—Hodgkin's disease is characterised by chronic enlargement of the organs of the hæmopoietic system with formation of special granulation tissue. It starts usually in the cervical glands. The cervical, retroperitoneal, mesenteric, and inguinal lymph glands are commonly affected. The enlarged glands remain soft at the beginning but as they become enlarged they become more and more dense in consistency. Although they may attain a huge size, there is little or no tendency of matting up or to show caseative or suppurative characters, although necrosis may occasionally take place. Similar changes occur in the spleen where the Malpighian bodies become enlarged, either in patches or diffuse throughout the organ. The bone marrow shows characteristic nodular appearance with both erythroblastic and leucobastic reactions.

Histology.—It consists of a large number of small and large round cells (lymphocytes). Proliferation of epitheloid cells with abundance of protoplasm and reticulum cells takes place. Eosinophilic leucocytes, numerous fibroblasts and very often Sternberg giant cells are the characteristic findings. The Sternberg giant cells are uninucleated or binucleated giant cells only rarely being multinucleated. The cells are big resemble megakaryocytes and are often seen to be connected with neighbouring cells by fine protoplasmic processes. Plasma cells are also occasionally present. The old nodules often show necrotic changes. Fibrosis or hyaline changes are also frequent. The microscopical changes in spleen and other organs are essentially the same as those found in the lymph nodes.

RHINOSCLEROMA

Rhinoscleroma is an infective granuloma characterised by a growth, fairly hard in consistency, on the mucous membrane and the adjacent skin of the nose and throat and rarely of the trachea and bronchi. *Macroscopically* it shows a nodular appearance and grows very slowly. *Histologically,* large number of plasma cells and thickened and proliferated collagen fibres with abundance of blood vessels are the characteristic findings. The skin and mucous membrane generally show atrophic changes. Hydropic and hyaline degeneration of the cells are common.

CHAPTER XXVIII

PROGRESSIVE TISSUE CHANGES

Hypertrophy.—This is a condition in which there is uniform overgrowth of a part with an increase in the actual size of its component tissue elements and a corresponding increase in functional activity. *Hyperplasia.*—This is a condition in which there is overgrowth of some particular tissue elements; it is commonly the result of reparative processes of the tissue after injury. *Heteroplasia.*—This is a condition in which there is overgrowth of abnormal tissue in place of the normal structure, *e.g.*, the formation of scar tissue. *Metaplasia.*—This is a condition in which there is growth of different type of tissue from that normally found in a structure, *e.g.*, chondroma growing from the salivary glands or the testes.

TUMOUR

Etiology.

A tumour is a new growth of tissue which is functionless and often harmful and has a tendency to persist and to increase. It is differentiated from hypertrophy which means uniform development of the tissue with corresponding increase of the physiological function. It is also differentiated from inflammatory new growth which develop under the influence of a definite source of irritation and terminate in a typical process of absorption and formation of fibro-connective tissue.

Predisposing causes:—(1) *Age.*—Age has a great influence as regards the occurrence of tumour. Thus, of the malignant tumours, carcinoma is an affection of the old age, generally after 40 years, whereas sarcoma generally occurs in the ealier periods but may occur at all ages. (2) *Sex.*—Both sexes are liable to the same condition as regards the growth of tumours; but in the females there are some organs, *e.g.*, uterus, mammary glands, etc., which are most functionating and most liable to be the seats of the growths. (3) *Occupation.*—The so-called carcinoma and sarcoma of chimney-sweepers are entirely due to the peculiarity of their occupation and habits. (4) *Heredity.*—It has been observed that certain families are more prone to a carcinomatous condition than others. (5) *Geographical* *distribution.*—Carcinoma is more common in low-lying damp places and is found all over the world.

Exciting causes. 1. *Irritation theory of Virchow.*—It has been observed that tumours grow commonly after some injury or irritation. The following may be cited as examples:—

(*a*) Sarcoma developing after traumatic injuries. (*b*) Adenoma of the breast following injuries. (*c*) Carcinoma of the tongue through irritation of carious teeth. (*d*) Carcinoma of lips from irritation of clay pipes. (*e*) Carcinoma of the abdominal wall of the inhabitants of Kashmir from irritation from heat of the smouldering of charcoal which they carry near their chest wall and between their thighs. (*f*) Carcinoma of the bile ducts and gall bladder secondary to the irritation caused by gall stones. (*g*) Carcinoma of the uterus is more common in mothers of many children. (*h*) Carcinoma is more common in tissues which are liable to friction or injuries, *e.g.*, mouth, cardiac and pyloric ends of stomach, rectum, cervix of the uterus, breast, etc.

2. *Theory of altered tissue resistance of Ribbert.*—Ribbert believes that there is a constant and continued tendency of any cell to grow but this process is normally controlled by the inhibitory influences of different types of cells. Thus, the epithelium is always tending to grow deeper and deeper and is controlled by the subjacent connective tissue. Whenever there is any deficiency of resistance of one of the tissue elements, the other will gain the upper hand and will grow, displace and invade into the former. This is exemplified in the case of epithelioma, papilloma, etc.

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3. *Theory of fœtal cell rest or vestiges* of fœtal organs developing into tumours. Conheim believes that embryonic cells are often displaced and deposited in various organs of the body and in this condition they can remain latent for years until some favourable condition arises when they multiply

with the formation of a tumour. As for example, it is believed that some of the adrenal tissue is retained in the kidney and remains latent for years and may proliferate at any time to such an extent as to lead to a more or less malignant character forming what is known as hypernephroma of the kidneys. Sutton believes that remnants of fœtal organs, *e.g.*, the thyrolingual duct, postanal gut, urachus, etc., often fail to disappear resulting in the formation of tumours and cysts subsequently.

4. *Parasitic theory.*—The malignant growths often resemble infective diseases and hence they are often believed to be due to some form of infection; the more so, on account of their metastatic growths and successful transplantation to healthy animals. Various writers claim to have demonstrated various forms of parasites both vegetable and animal in tumours but all of them lack in confirmation. The parasitic theory is nicely summarised by McFarland in the following words:—" From this review of the literature it will be evident that with the evolution of parasitology theories regarding the infectiousness of carcinoma have kept pace. When men were most interested in the study of bacteria, they found them in the tumours; when protozoa were shown to infect the epithelial cells of the lower animals, and their attention was turned to the sporozoa, they naturally looked for them in carcinoma; and when the blastomycetes were found to be of some importance, they were not slow to produce yeasts cultivated from tumours. From the evidences presented we are led to the conclusion that the parasitic nature of carcinoma is not proved."

5. *The gene theory.*—The human cell, whether the germ cell or the somatic cell contains 48 chromosomes, 24 of maternal and 24 of paternal origin. Each chromosome contains many hundreds of minute living particles called genes. At the present time the concept of the gene is of great biological importance and the gene occupies much the same position as the atom in physics. The chromosomes are regarded as the physical basis of heredity and the genes the controlling factor in the behaviour of the cell. The genes are very minute and have been estimated to measure $20\text{-}77\mu\mu$, the human red cell being $7500\mu\mu$.

During cell division each chromosome splits longitudinally leaving the same number of the genes in each daughter cell as the parent one. It is the opinion of the biologists that accidental mutation of gene generally leads to the evolution of new species. This is the foundation of the gene theory of tumour formation.

The gene mutation of the sometic cell is thus believed to be the factor for the formation of tumours. This further explains the specificity of the tumour cells to the tissue of its origin and also why the tumour cells grow by their own division and not by converting the neighbouring cells into tumour cells. This gene mutation theory of production of cancer has been originated from the fact that malignancy can be produced by irradiation with the Roentgen rays.

6. *The Virus Theory.*—By this is assumed that an infective agent is present in tumours specially the malignant ones. In majority of instances it is specific, a new tumour originating from a tumour filtrate. Rous sarcoma maintains its specificity for years and the filtrate of the Rous sarcoma always produces a spindle cell and it has been found to have been doing so for more than 21 years.

Classification. The scientific classification of tumour is most unsatisfactory. Various authorities have attempted to find out a satisfactory method of classifying tumours but with only partial success Attempts have been made to classify on histogenetic principles as well as on the basis of their etiology. The classification of Virchow on the origin of tumours derived from one or more of the three blastodermic layers is more convenient. He classifies as follows:—

A. *Histoid or Histioid Tumours.*—These are tumours derived from one blastodermic layer and resemble simple tissues. (*a*) Of adult type *e.g.*, fibroma, myxoma, lipoma, chondroma, osteoma, myoma, etc. (*b*) Of embryonic type *e.g.*, sarcoma.

B. *Organoid Tumours.*—These are tumours derived form two blastodermic layers; *e.g.*, papilloma, adenoma, carcinoma, etc.

C. *Teratoid Tumours.*—These are tumours derived from all the three blastodermic layers; *e.g.*, teratoma, dermoids and some mixed parotid tumours.

Morphology of tumours. *Composition.*—The tumour is composed of the specific tissue of the host. It is regarded to be specific in nature, from the transplantation experiments, in which it has been found that a carcinoma of a white mouse, when transplanted, will grow only in another white mouse and not in a grey or wild mouse. or in a rat or other animal. Similarly transplantation of human tumours to other animals, *e.g.*, rabbit, dogs, even monkeys have failed. The tumour cells resemble very closely, the normal tissue histologically, but they are not exactly alike.

Size.—It varies from a pin's head to an enormous size. There is no limitation to the size. Deficiency of blood supply, infection and other conditions often interfere with its growth by destruction of the substance.

Shape.—The general shape of a tumour is spherical but may be of any shape according to its situation or pressure from contiguous structures or from gravity.

Consistency.—This depends upon its composition. Myxomatous and lipomatous tumours are very soft whereas osteoma or chondroma are exceedingly hard. Other tumours become abnormally soft owing to the degenerative processes in them or become harder due to the deposition of calcareous particles.

Number.—This is usually single but may be multiple and in cases of molluscum fibrosum may be more than one thousand in number.

Vascularity.—The new growth is supplied by simultaneous overgrowth of the pre-existing blood vessels which is never uniform. When the growth of the tumour is very rapid the new formation of the blood vessels cannot keep pace with the same and often falls short leading to degenerative changes owing to malnutrition. In some instances there may be excessive growth of the blood vessels resulting in increased vascularisation of the tumour. The tumour may be telangiectatic, when the blood vessels are abnormally large in size, or cavernous when it contains cavities full of blood.

There is usually a deficiency of capillaries interfering with the proper distribution of the blood supply. In sarcoma and other tumours the walls of the blood vessels are usually defective favouring the transference of the sarcomatous cells to other parts of the body through the blood stream causing what is known as metastasis. Tumours are richly supplied with lymphatics, but there are no nerves, and they are not under the control of the nervous system.

Disposition. The tumours are either benign or malignant according to their tendency to do harm to the host.

Benign tumours are characterised by the following:—

(1) Tendency to remain localised.

(2) No tendency to do any harm other than mechanically by pressure or peculiarity of its position. Thus a benign tumour may

lead to paralysis and often death by its mechanical pressure on the vital centres of the brain or spinal cord. A papilloma of the larynx may cause suffocation and death by its peculiar position inside a vital channel.

(3) The proportion and normal disposition of the various tissues remain intact with no tendency to disorganise tissue. The benign tumours are usually surrounded by a capsule which prevents infiltration of the cells into the neighbouring tissues.

(4) No tendency to have secondary growths or metastasis. The benign tumours do not form any cellular embolus in distant parts through circulation so that the deposited cells could multiply and form a secondary or metastatic growth in the new position.

(5) No tendency to recur after excision. As these tumours are well circumscribed and surrounded by a capsule, they can be excised *en masse* leaving no cell behind to grow and hence there is no recurrence of the tumour.

Malignant tumours are characterised by the following:—

(1) No tendency to remain localised but to infiltrate the neighbouring tissues. Thus there is no tendency for encapsulation.

(2) Tendency to do harm by absorbing the nourishment of the host and also by some toxic products leading to wasting, anæmia, cachexia and often death.

(3) Tendency to disorganise the tissues by the growth of the cells in the interstices of the neighbouring tissue or inside the blood or lymph vessels with subsequent obstruction.

(4) Tendency to have secondary growth or metastasis by transportation of the cells through the circulation to distant parts of the body where they grow and form new fresh growths.

(5) Tendency to recur after excision. As these growths are not encapsulated some of the tumour cells are apt to be left behind during operation which will grow and form a fresh growth resembling what has been excised.

THE CONNECTIVE TISSUE TUMOURS

The ordinary connective-tissue cell, the fibroblast, produces normally fibroglia, collagen fibril and in the walls of the blood vessels, elastic fibrils. Under abnormal conditions, it also produces mucin, chondromucin and osseomucin. Thus in the tumour formation, if the fibroblast produces only fibrils, the condition is known as fibroblastoma which according to the ripe or unripe stage is differentiated into fibroma and fibrosarcoma. Similarly, if the fibroblast produces both fibrils and gelatinous homogeneous intercellular structure, the condition is called myxoblastoma including myxoma or myxosarcoma according to the ripeness of the cells. Similarly if chondromucin or osseomucin with deposition of lime-salts are produced by the fibroblast, chondroblastoma or osteoblastoma is formed; the collagen fibrils, cartilage and bone cells are the end products of the fibroblast, and so no further change of the structure takes place and these cells by mitosis produce the adult type of the tissue.

On account of the production of these types of cells from the fibroblast it is easily conceivable how the various combinations may occur, *e.g.*, fibro-chondro-myxo-osteoma or even fibro-myxo-chondro osteosarcoma.

FIBROMA

Fibroma is a tumour composed of tissue resembling fibroconnective tissue, and tend to produce fibroglia and collagen fibrils.

Seat of occurrence. It occurs almost in every part of the body most commonly in skin, subcutaneous tissue, fascia, intermuscular septum and submucous tissue. It also originates from periosteum, about the joints, bones, nerves, etc. Fibroma are very common in connection with the ovary, uterus and the breast in which condition they exist as fibromyoma or fibro-adenoma.

Morbid anatomy. *Size.*—Varies from a pin-head to an enormous size weighing several pounds. They are generally nodular

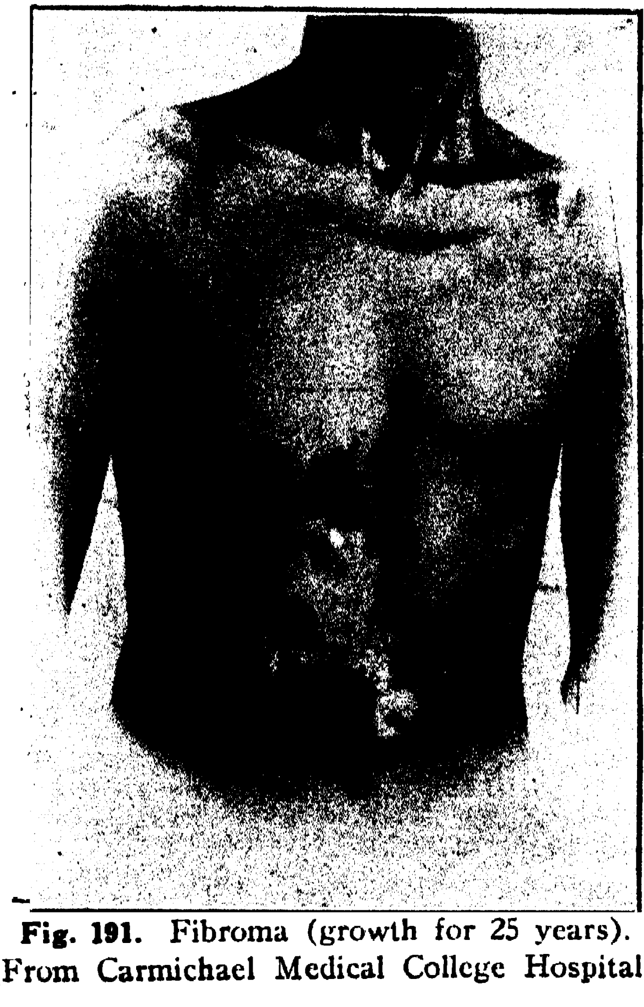


Fig. 191. Fibroma (growth for 25 years). (From Carmichael Medical College Hospital).

but may be polypoid due to the presence of a short or long pedicle. *Number.*—May be single or multiple. In fibrosum molluscum there may be several thousands of the soft nodules scattered all over the body. *Disposition.*—They are benign, growing slowly, well-encapsulated and sharply defined. *Consistency.*—Some are soft, others are hard and solid. *Section.*—Bands of whitish fibrous tissue are distinctly visible with the naked eye.

Pathological histology. It consists of fibrils and cells resembling normal fibro-connective tissue but varies a good deal as regards the arrangements of the fibres which are not always regular and have no relation to the neighbouring tissue. The fibrils are spread throughout the tumour in masses and also in every direction in a purposeless manner. In some instances the tumour is very soft and consists of cells mainly, with few or no fibres. The section resembles one of spindle celled sarcoma, which Borst differentiates by the presence of—(*a*) irregular size and shape of the cells, (*b*) overgrowth of the nucleus, and (*c*) absence of a definite stroma.

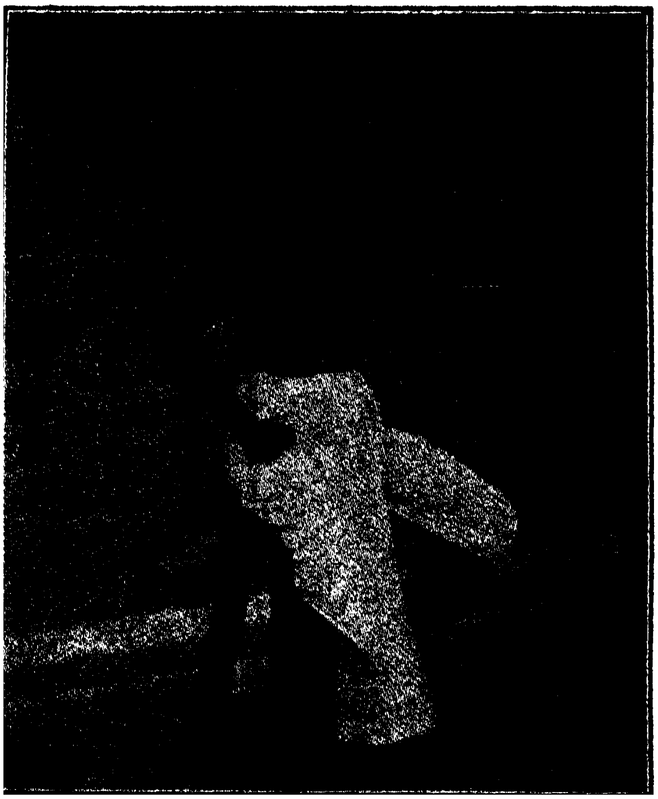


Fig. 192. Gynomastica.
(From Dr. Charubrata Roy's collection).

Retrogressive changes. On account of irregular blood supply, necrosis is a common feature. Interstitial hæmorrhage and hence pigmentation are common. Calcification and myxomatous change occur frequently. Fatty changes are rare.

Varieties. *Keloid.*—It originates from scar tissue and is composed of extremely hard and dense fibrous tissue. It usually originates from scars following burns and is common among the Negroes. Keloids grow slowly and do not occur in all persons. They often recur after excision due to repetition of the same process from the scar following the excision. Histologically it consists of a thick, hyaline matrix including masses of fibroblasts.

Fibrosum Molluscum.—This is a fibromatous condition of the skin in which the fibrous tissue is deposited in the subcutaneous tissue with hyperplasia of the skin as well. They are mutiple, often amounting to several hundreds in number and cover the whole surface of the body. They vary in size to a wide extent, the large ones being polypoid. The affection is generally seen in the adult life but may be congenital. They may be pigmented.

Histologically.—It consists of fibrous and fatty tissue with presence of nerve fibres in them which are usually found in all stages of atrophy.

Papillary Fibroma.—A fibroma lined by hypertrophic epithelium.

Adeno-fibroma.—A fibroma mixed with secretory glandular structure.

Neurofibroma.—A fibroma originating in connection with a nerve trunk.

Angio-fibroma.—A fibroma with abundant blood supply.

Mixed Fibroma.—These are fibro-lipoma, fibro-myxoma, etc.

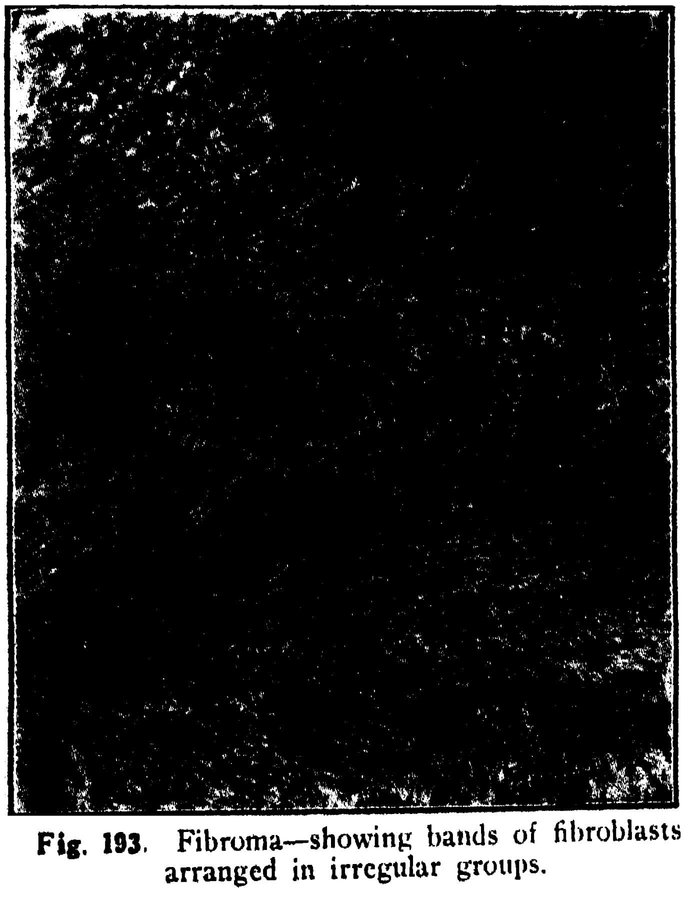


Fig. 193. Fibroma—showing bands of fibroblasts arranged in irregular groups.

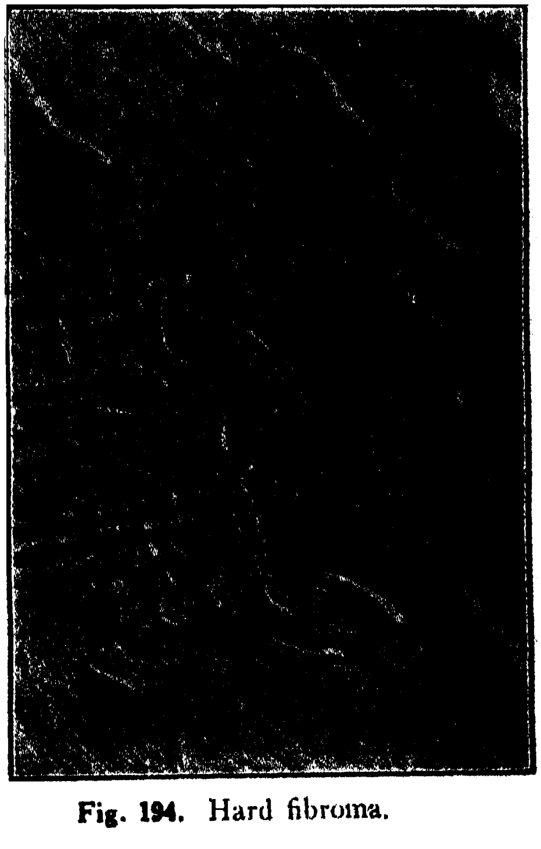


Fig. 194. Hard fibroma.

MYXOMA

Myxoma is a tumour composed of mucoid tissue. This originates from the connective-tissue cell which tends to produce mucin, fibroglia, collagen and elastic fibrils. True myxoma is rare. It is nearly always found as the mixed form, *e.g.*, fibro-myxoma, chondromyxoma, etc.

Seats of occurrence.—The subcutaneous, submucous and intermuscular tissue specially in those places where the structure is fatty and loose. The regions affected are—the thigh, neck, leg, peritoneum, bones, nerves, meninges, mucous membranes, etc.

Morbid anatomy.—Myxomata are soft, lobulated, polypoid, encapsulated growths.

Disposition.—They grow slowly and are benign in nature. Myxomata of striated muscles, fascia and certain organs are usually malignant in nature resembling sarcoma.

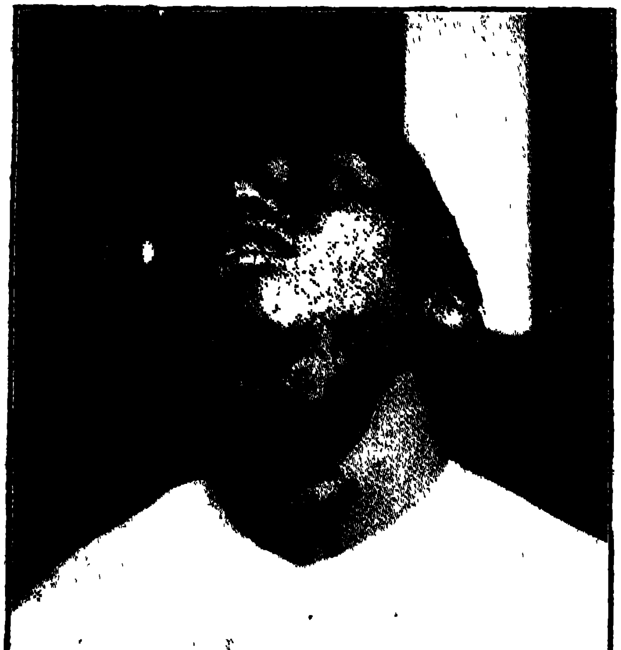


Fig. 195. Keloid of the neck. (From Dr. P. K. Mitra)

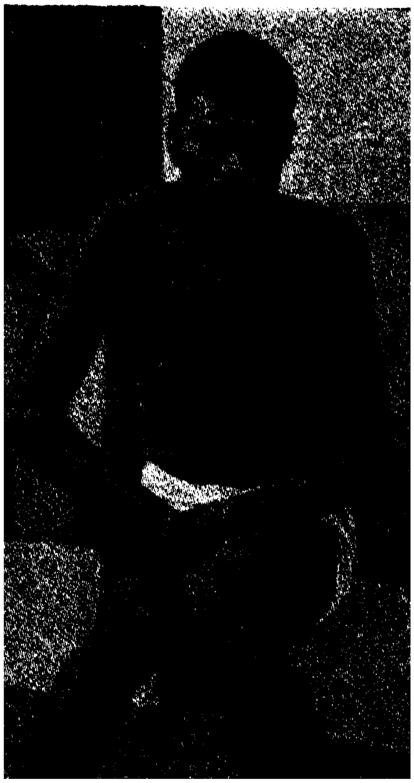


Fig. 196. Multiple Keloids.

Histology.—The structure consists of star-shaped cells with processes which anastomose freely. The interstices are filled up with opalescent, viscid, mucoid and watery materials. They occasionally become œdematous by absorption of water. Myxomatous tissue is usually combined with other tissue elements, *e.g.*, fat cells, cartilage, bone, fibrous tissue, etc.. This tissue is not found in the adult. The Wharton's jelly of the umbilical cord contains this tissue normally.

Chemistry.—The mucus is dissolved by weak alkali and is precipitated by acetic acid. It is also precipitated by alcohol in membranous or reticulated form.

LIPOMA

Lipoma is a tumour composed of fatty tissue.

Seat of occurrence.—Lipomata usually grow in the subcutaneous tissue, the commonest site being the back, neck, shoulders, axilla, abdominal wall, etc. They are usually found in the dermis of the skin, fascia, submucous, subserous, subsynovial, intermuscular, perirenal and other tissues.

Dercum's disease or *adiposis dolorosa* is a condition characterised by the presence of numerous symmetrical lipomata which are often very painful and tender. Various nervous and trophic disturbances are also associated with this condition.

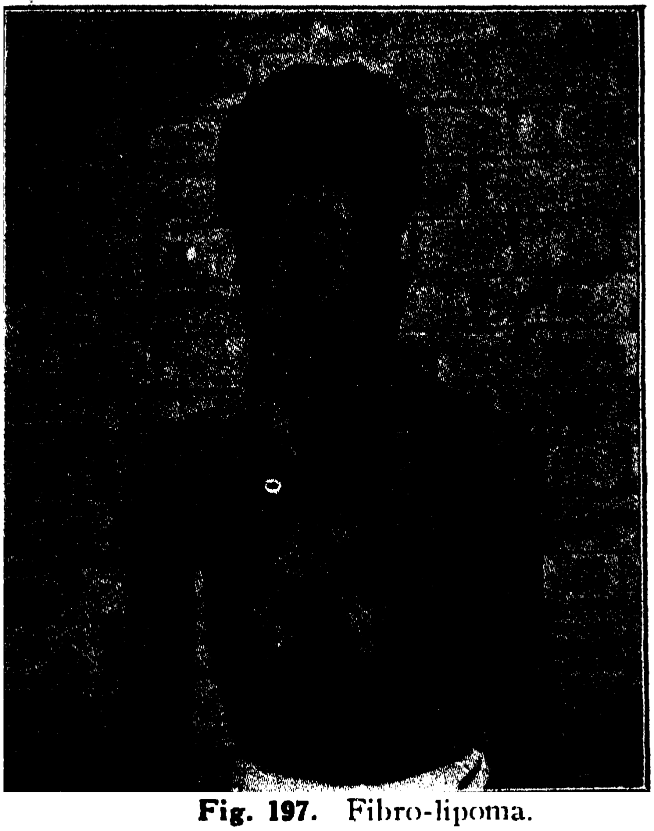


Fig. 197. Fibro-lipoma.
(From Dr. Charubrata Roy's collection).

Morbid anatomy.—Lipoma is a soft, elastic, lobulated, well-encapsulated, sharply circumscribed growth. The size varies from that of a pea to an enormous size weighing several pounds, frequently causing serious inconvenience due to pressure and weight. The soft consistency depends on the amount of the fatty tissue present but becomes firm due to the presence of fibrous or other tissues. It is yellow in colour resembling normal fat, on section the knife is soiled with fat. Lipomata are richly supplied with blood and usually grow in connection with a big vessel. Large lipomata occasionally absorb so much nutrition of the host as to induce emaciation. It is a peculiar feature to note that during emaciation of the individual, as in the case of pulmonary tuberculosis, etc., the fat in the lipoma remains unaffected and occasionally continues to grow inspite of the wasting of the individual. Lipomata never recur after excision.

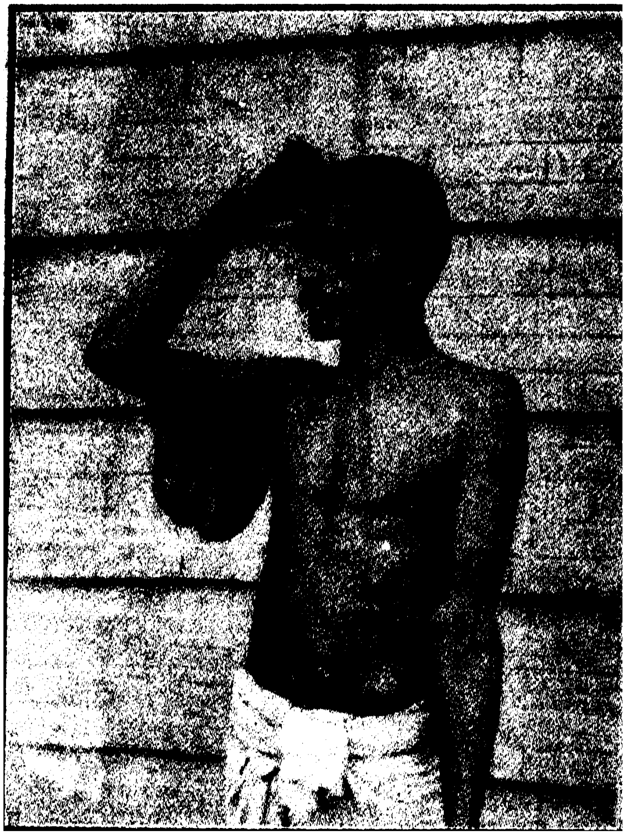


Fig. 198. Fibrolipoma.
(From Dr. D. C. Chakrabarty).

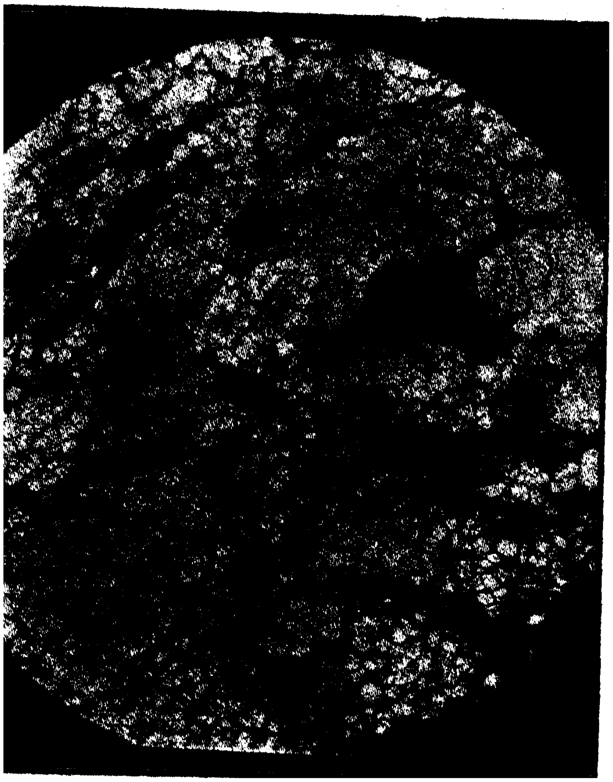


Fig. 199. Lipoma—Showing
(*a*) Numerous fat cells.
(*b*) Few bands of fibrous tissue.

Histology.—Under the microscope a lipoma resembles normal fatty tissue. The cells are distended with fat, nucleus displaced to one side and supported by irregularly arranged stroma. It is rare to find coalescence of the fat-cells with loss of the wall.

CHONDROMA

Chondroma is a tumour composed of cartilaginous tissue.

Seat of occurrence.—It grows either in connection with pre-existing cartilage (ecchondroma) as in the ribs, larynx, about joints, etc., or in tissues not containing cartilage normally (enchondroma) as in salivary glands, testes, breast, etc. Its growth as multiple masses in the pelvis causes obstruction to child birth. In other places, it renders the part, *e.g.*, the hand, joints, etc., more or less useless.

Morbid anatomy.—These may be single or multiple and occasionally assume a huge size. They are hard, lobulated, well encapsulated, grow more or less slowly and are benign in nature. Although they are benign they are often seen to grow inside blood vessels and fill up their lumen. There are few blood vessels inside the tumour, the nutrition being mainly managed from the vessels in the capsule.

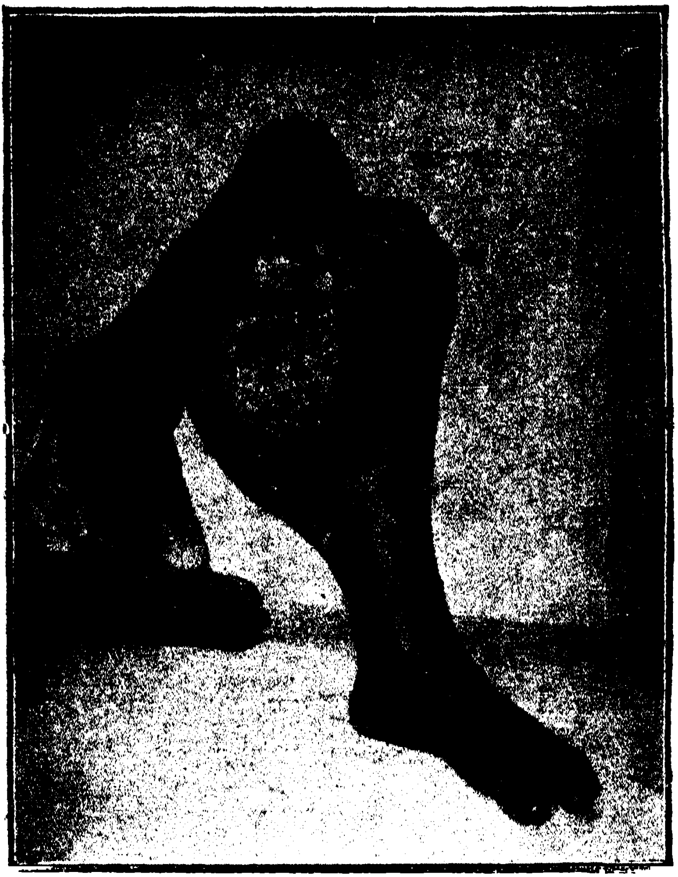


Fig. 200. Chondroma leg
(From Carmichael Medical College Hospital)

Histology.—The structure of a chondroma consists of cartilaginous tissue and resembles more or less normal type. Thus they may be hyaline or fibro-cartilaginous, either pure or both forms mixed up.

Varieties.—*Ecchondroma*.—They are usually small, smooth or nodular, multiple growths from the pre-existing cartilage. After a certain amount of growth they may be ossified or calcified. They cause great inconvenience when growing in connection with the cartilage of joints or of those of the larynx, trachea, intervertebral discs, etc. Chondroma growing from the inner side of the symphysis pubis may obstruct labour.

Enchondroma.—They are fairly common and are large, lobulated more or less rounded growths from structures containing no cartilage normally. They may assume a huge size. They are well encapsuled by perichondrium. The capsule of sub-periosteal chondroma is chiefly derived from the periosteum which may ultimately lead to the formation of bone rendering the whole capsule bony.

Mixed Chondroma.—These occur according to the associated element present with the cartilage cells. They are as follows: Fibrochondroma, myxochondroma, osteochondroma, chondrosarcoma, etc.

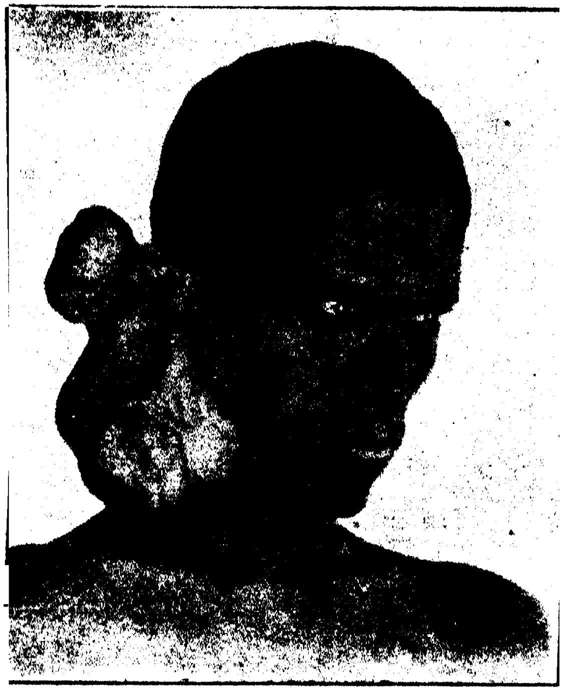


Fig. 201. Chondroma right ear. (From Carmichael Medical College Hospital).

Fig. 202. Chondroma.

Degeneration.—The chondroma may be soft through mucinous or cystic degenerations. The skin over the chondroma becomes atrophied and ultimately ulcerates with evacuation of some of the tumour mass. Calcification or ossification may take place.

CHORDOMA

This is a form of growth originating from the remnant of the notochordal tissue—the embryonal tissue about which the spinal column develops. It is composed of groups of large vacuolated cartilage cells lying in soft homogenous matrix. They occasionally assume a malignant character.

OSTEOMA

Osteoma is a tumour of osseous tissue. This must be differentiated from calcification which originates from the deposition of calcareous particles in the tissues, *e.g.*, in the necrotic tissue, base of ulcers, tuberculous lesions, after fractures, in chronic arthritis, etc.

Seat of occurrence.—The commonest sites are the long bones, vertebræ, pelvis, scapula and the skull. They also grow inside the joints or in the marrow cavity.

Morbid anatomy.—These tumours are usually single but may be multiple and grow slowly. In case of slow growth they require several years to attain some size. The tumours are exceedingly hard, well encapsulated, rounded, smooth or irregular, sessile masses.

Morbid histology.—The microscopical structure consists of dense laminated bone with few Haversian systems. In case of spongy bone there may be numerous blood vessels with wide marrow spaces containing cells. Numerous osteoblasts may be seen surrounding the edges of the growth adding new bony tissue to it.

Varieties.—*Exostosis.*—These are circumscribed tumour-like masses projecting above the bony surface. They may be flat, nodular, pointed, rough growths on the surface of the shafts of the bones, epiphyseal ends or at the insertions of the tendons or fascia. They are of two varieties:—(*a*) Ivory exostosis—these are flat, smooth, very hard, ivory-like growths originating from the skull, etc. (*b*) Cancellous or spongy exostosis—these are usually multiple and porous in structure.

Enostosis.—These are growths originating from inside the marrow cavity of the long bones or within cancellous bones.

Hyperostosis.—These are diffuse enlargement of bones, *e.g.*, in acromegaly.

Osteophytes.—These are sub-periosteal bony deposits of inflammatory origin. They appear as flat, plate-like, circumscribed, detachable masses developing on the surface of pre-existing bony tissue. They usually occur on the shafts of long bones, in chronic arthritis, on the jaws round inflamed teeth and in the neighbourhood of chronic ulcers and sinuses. They grow progressively so long as the inflammation lasts, the further progress being checked when the inflammatory process subsides.

Microscopically.—The periosteum is found to be thickened, the cellular and vascular cancellous tissue contains thin trabecula of bone in their meshes.

Myositis Ossificans is the formation of spongy bony tissue in the muscles most liable to trauma especially of the working people

They have been found in the biceps, neck muscles and muscles of the thigh. In some instances they may be very extensive affecting the face, neck, shoulder, trunk and limbs. There may be pain and local congestion in the early stages.

ODONTOMA

These are tumours growing from the teeth either from the enamel, cement or the dentin. Sutton describes the different forms according to their origin as follows:—

Epithelial odontome--originating from the enamel.
Follicular odontome or dentigerous cyst ..
Fibrous odontome .. ..
Cymentome .. .. ..
Compound follicular odontome ..
} Originating from the tooth follicle.
Radicular odontome—Originating from the roots of teeth.
Composite odontome—Originating from all the elements of the tooth germ—enamel, dentin, cement, follicle, etc.

MYELOMA

Myeloma is a tumour growing from the bone marrow. Formerly it was called myeloid sarcoma but it has been recently realised that it should be grouped as a benign tumour derived from cells of the bone marrow and termed as myeloma. That it may on some rare occasions take a malignant turn is explained by assuming that in those cases it behaves like benign growths which may occasionally turn towards malignancy.

Seat of occurrence.—They are common in the upper end of humerus, lower end of radius, upper end of tibia, lower end of femur and lower jaw. They are rare in other situations.

Morbid anatomy.—The tumour originates from the bone marrow, the bone thereby becomes expanded with absorption in places. The absorption of bone renders it very thin and hence the clinical sign " egg-shell crackling." There is no infiltration of the tumour cells in the surrounding structures. They are very vascular and are liable to hæmorrhages.

Histology.—It consists of a large number of cells resembling those of sarcoma with numerous giant cells. The giant cells may be few or numerous, they may be small or large, they may contain only a few nuclei or the nuclei may be very numerous. The nuclei are placed in the central part of the giant cells in a mass, like osteoclasts (*compare* the crescentic arrangement of the nuclei in tuberculosis).

Disposition.—They are benign in nature. Occurrence of metastasis is rare. The disorganisation of the bones renders them liable to fracture and other accidents.

Multiple Myeloma or Myelomatosis

These are multiple tumours and occur in various parts of the body chiefly ribs, sternum, vertebra and cranium. They orginate from the red bone marrow causing absorption of bone and often induce fracture.

Histology.—They are composed of those cells only, which are believed to be identical with Unna's plasma cells. Bence-Jones protein, a form of albumose, is found in urine in considerable amount.

MYOMA

Myoma is a tumour composed of muscle tissue. There are two types of myomata:—(*i*) Leiomyoma—This is composed of unstriped muscle fibres. (*ii*) Rhabdomyoma—Composed of striped muscle fibres.

LEIOMYOMA

This is usually associated with hyperplasia of fibrous tissue as well.

Seat of occurrence.—The uterus is the commonest seat. These are usually multiple and affect in order of frequency its posterior wall, the anterior wall, lateral wall and the cervix.It also affects the broad ligament, vagina, pelvic fascia and other parts. In the uterus they are often loosely termed fibroids and they may be subserous, interstitial or submucous. Leiomyoma also occurs in connection with the stomach, intestines, skin, urinary bladder, kidney, etc.

Morbid anatomy.—Leiomyomata occur as single or multiple, small or large, dense solid growths often becoming soft due to cystic or retrogressive changes. They grow slowly, they are well encapsulated, well circumscribed and benign in nature. They are often found as pedunculated growths

Histology.—The unstriped muscle fibres run in parallel rows in interlacing bundles, in all directions supported by numerous bands of fibrous tissue and blood vessels. The muscle-fibres possess rod-shaped nuclei, the cell-body is plump, and takes yellowish colour with

Van Gieson's stain. The fibrous stroma takes bright red colour with Van Gieson.

Retrogressive changes.—Are very common. Fatty degeneration, hyaline degeneration, calcification or cystic changes often take place. Infection and inflammatory changes often lead to necrosis and suppuration.

RHABDOMYOMA

This is of embryonic origin and is a rare form of tumour. It usually occurs in connection with other tumours especially the sarcoma. It is usually congenital or occurs in early life.

Seat of occurrence.—The genito-urinary tract specially the kidney is the commonest site. It also occurs in the stomach, breast, prostate and skeletal muscles of various parts of the body.

Morbid anatomy.—They are usually single or multiple, nodular, rounded diffuse growths. They grow rapidly and are malignant in character.

Histology.—The structure consists of bundles of interlacing striped muscle fibres running in all directions. The cells of rhabdomyoma present an embryonal character in being thin and having much elongated fibres and rarely possess adult character.

LYMPHOMA

This is a tumour originating from the lymphoid tissue. Lymphoma although very difficult to differentiate histologically, is quite distinct from hypertrophy, hyperplasia and similar conditions of the lymph gland. The present state of our knowledge is still quite imperfect as regards the true nature of the different conditions grouped under this head. Affections of the lymphoid tissue may be localised to the lymph nodes only but may extend to the other leucopoietic system as the spleen and bone marrow. Thus all grades of affections are possible which may be benign or even malignant.

The following, *e.g.*, lymphatic leukæmia, pseudoleukæmia, small round celled sarcoma, Hodgkin's disease, and lymphosarcoma, are also used synonymously with the lymphoma. This variety of names are due to the lack of distinctive characteristics by means of which they can be identified exactly. Hodgkin's disease gives some definite histological picture of a granuloma and not of a tumour. Strictly speaking, it is lymphogranuloma and should not be classified as lymphoma or lymphadenoma.

SARCOMA

Sarcoma is a malignant tumour composed of mesoblastic cells of embryonic type.

Seat of occurrence.—Sarcoma occurs in every part of the body wherever connective tissue is present.

Disposition.—Sarcoma possesses all the characters of a malignant tumour.

Morbid anatomy.—Sarcoma is a bulky, soft, rapidly growing vascular tumour. It is usually single but may be multiple both in the primary or in the secondary metastasis. The growth is rapid due to the extensive and rapid multiplication of the cells which grow and expand the surrounding tissue. This rapid cell growth is often favoured by rich blood supply of the part. Sudden increase in bulk also occurs due to hæmorrhage, œdema, etc. The growth of blood vessels cannot keep pace with that of the cells and so sooner or later blood supply falls short leading to necrosis and other degenerative processes. Sarcoma in such a necrosed condition is very liable to be infected and followed by severe septicæmia.

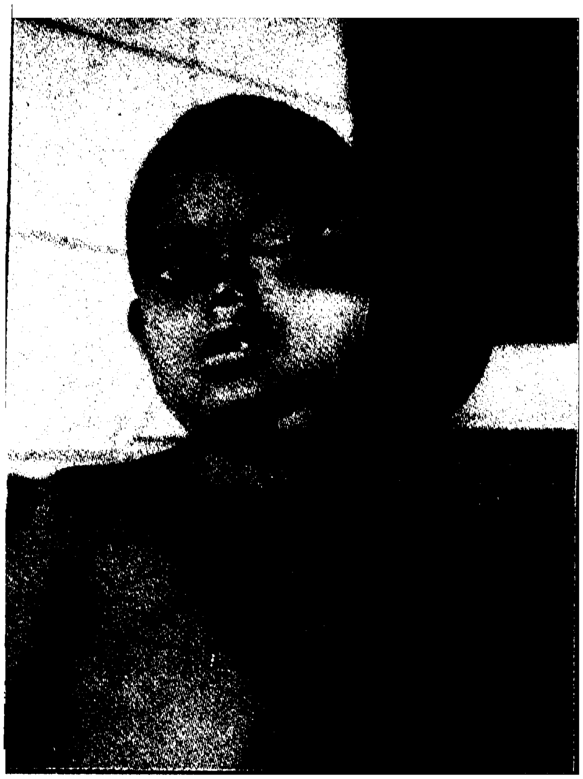


Fig. 203. Sarcoma upper jaw. (From Dr. N. N. Das's collection).

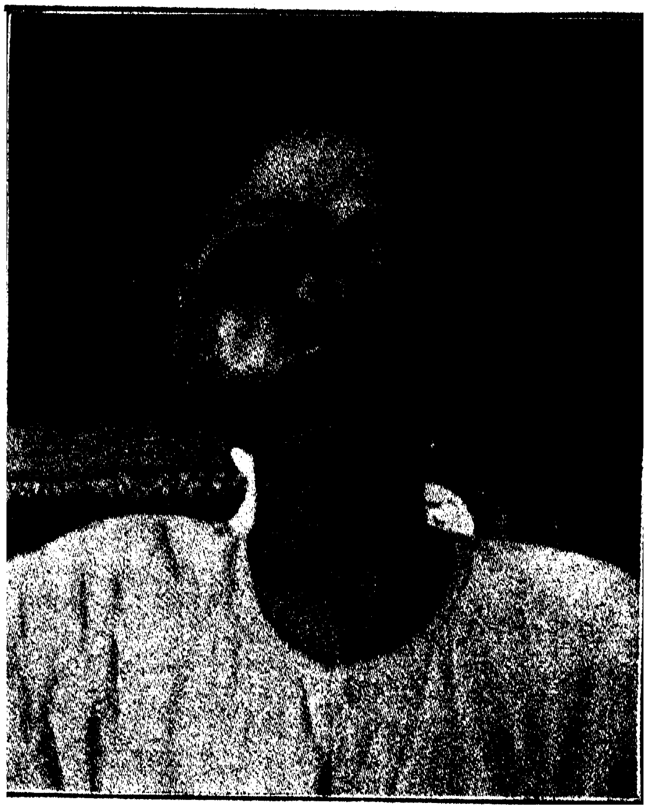


Fig. 204. Sarcoma of the upper jaw. (From Dr. Nagendra Nath Das's collection).

As the cells grow and the tumour is expanded most sarcomata remain well defined but a capsule is wanting. Hence cells infiltrate into the surrounding structures. Thus complete removal is difficult and is liable to recurrence after excision.

The cells often grow inside the lumen of blood vessels, and are carried to distant parts of the body leading to secondary metastatic growths in different parts of the body. Unlike carcinoma the sarcomatous tissue contains little lymphatics rendering lymphogenic matastasis rare. The veins, being thin walled than the arteries, are readily infiltrated and so the sarcoma cells are more easily carried to the lungs than other structures. The

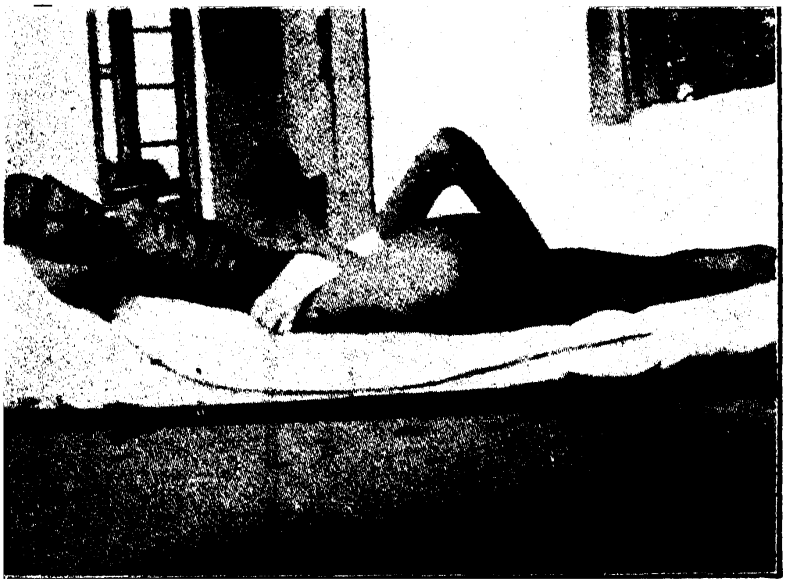


Fig. 205. Sarcoma right thigh in a boy of 16 years. (From Carmichael Medical College Hospital).

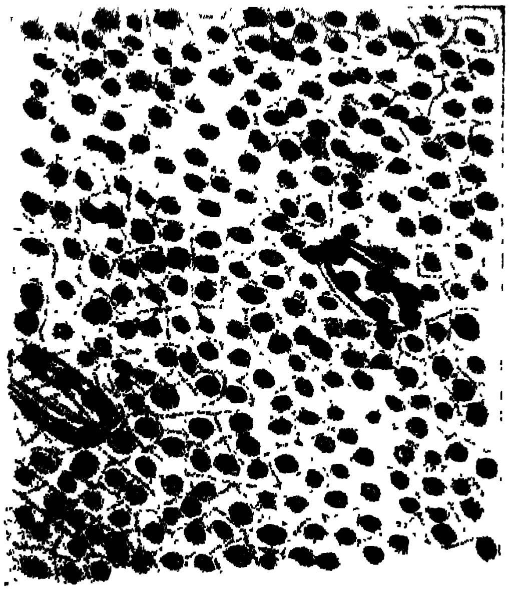


Fig. 206. Round-celled Sarcoma.

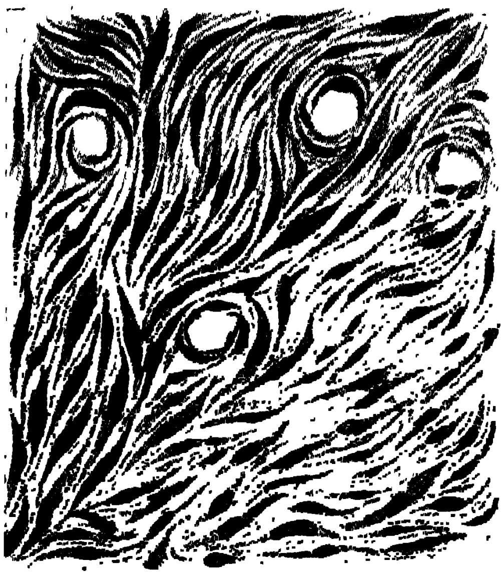


Fig. 207. Spindle-celled Sarcoma.

secondary growth always resembles closely the structure of the primary one.

Histology.—A sarcoma is composed of cells and supporting framework or stroma composed of fibrous tissue and blood vessels. In sarcoma each individual cell is being held by a delicate band of fibrous tissue, so that a layer of fibrous tissue exists between any two adjacent cells. This sort of arrangement distinguishes sarcoma from cancer as in the latter fibrous tissue is present as bands in the form of alveoli with no intercellular material between individual cells.

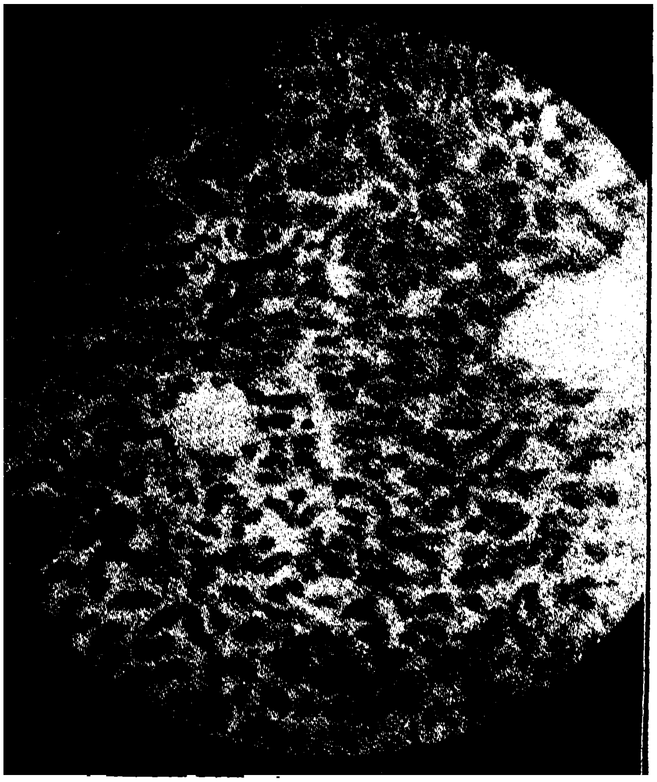


Fig. 208. Round-celled sarcoma with defective walls of blood-vessels.

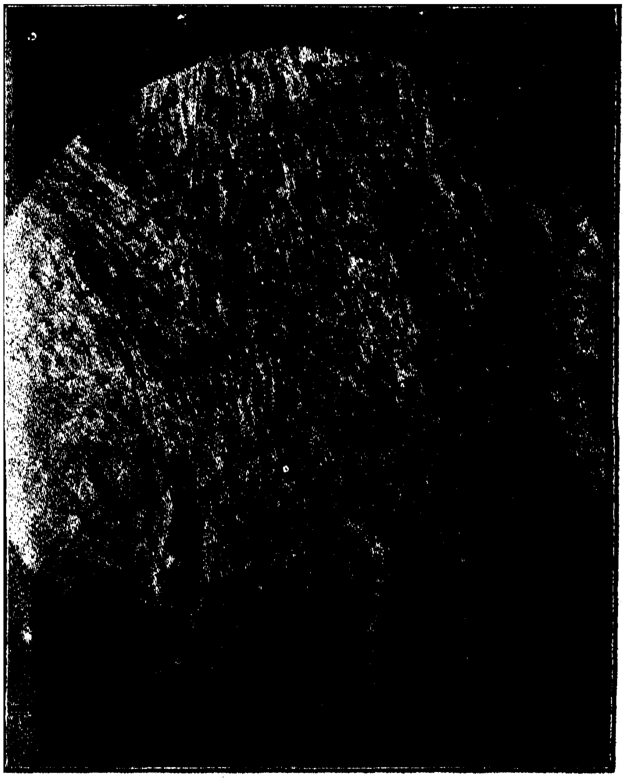


Fig. 209. Spindle-celled Sarcoma.

The blood vessels are abundant and exist as spaces in the tumour mass. The walls of the vessels being deficient to a great extent occasionally allow the tumour cells to grow inside the lumen and thus favour metastasis through blood as emboli. The sarcoma cells never show any tendency to specialise into the adult type of cell.

Varieties of Sarcoma

Round-Celled Sarcoma.—Consists of a mass of round cells with a round nucleus often showing karyokinetic changes. Each cell is being held by a delicate band of fibrous tissue. They are very malignant with early deposition of secondary growths. Round-celled sarcoma may be of two types according to the type of cells present, *e.g.*, (1) Small round celled sarcoma and (2) Large round celled sarcoma.

Lymphosarcoma.—Is a form of small round-celled sarcoma resembling the structure of a lymphatic gland in which it takes its origin and grows very rapidly. This form is disseminated by means of the lymphatics. Large round celled sarcoma is less malignant than the former variety.

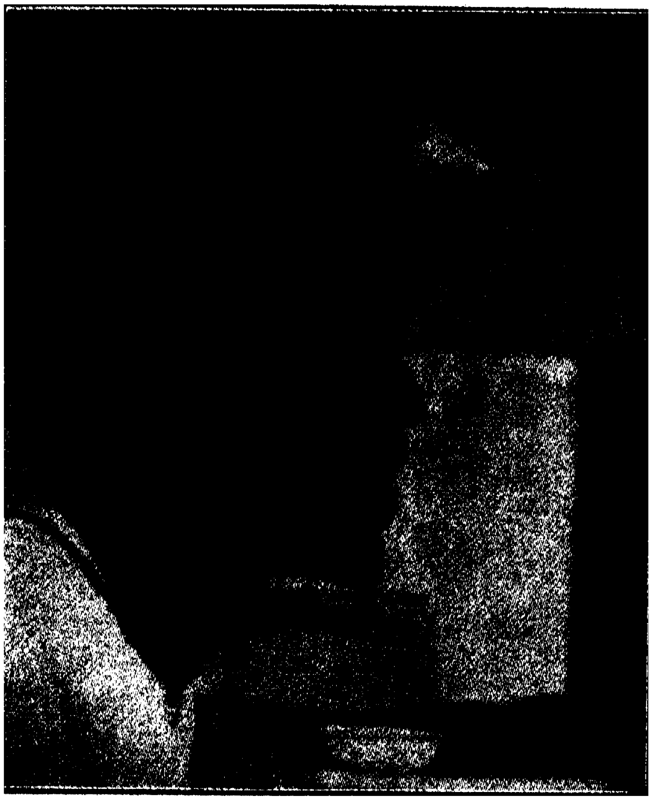


Fig. 210. Sarcoma forehead. (From Dr. P. K. Mitra).

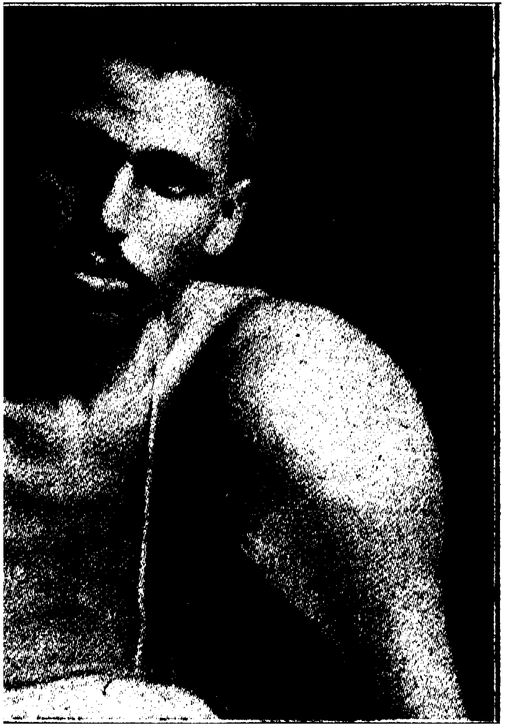


Fig. 211. Sarcoma arm. (From Dr. P. K. Mitra)

Small Round Celled Sarcoma.—The cells are small and spherical, with deeply staining nucleus and scanty protoplasm. The intercellular substance is very scanty and shows occasional fibrils. Blood vessels are fairly large in number with defective walls.

Seat of Occurrence.—May occur in any part of the body. It occurs most frequently in the subcutaneous tissue with secondary growths in the lungs, liver, spleen and other organs.

Large Round Celled Sarcoma. The cells are bigger, nuclei stain less deeply and the protoplasm is more abundant. The intercellular substance shows numerous fibrils often with alveolar arrangement.

The blood vessels are less numerous and the walls are better supported.

Seat of Occurrence.—They occur in the skin, muscles, ovary and testes.

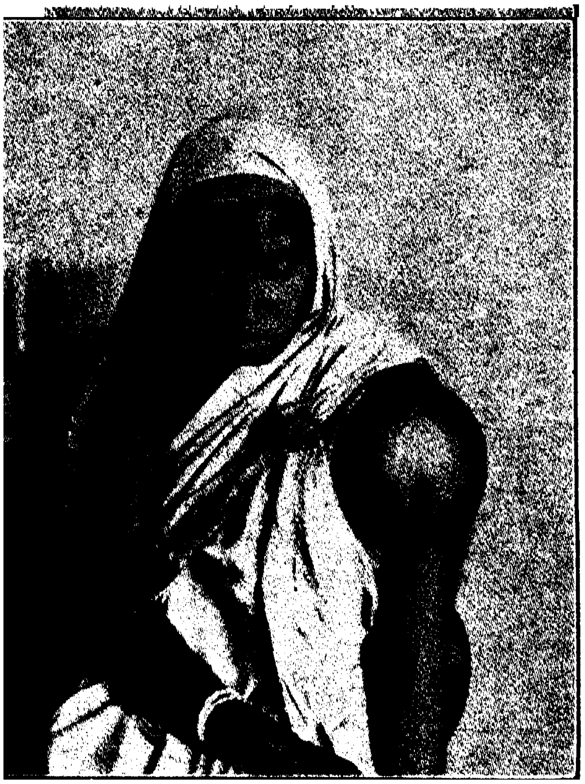


Fig. 212. Sarcoma arm.
(From Carmichael Medical College Hospital).

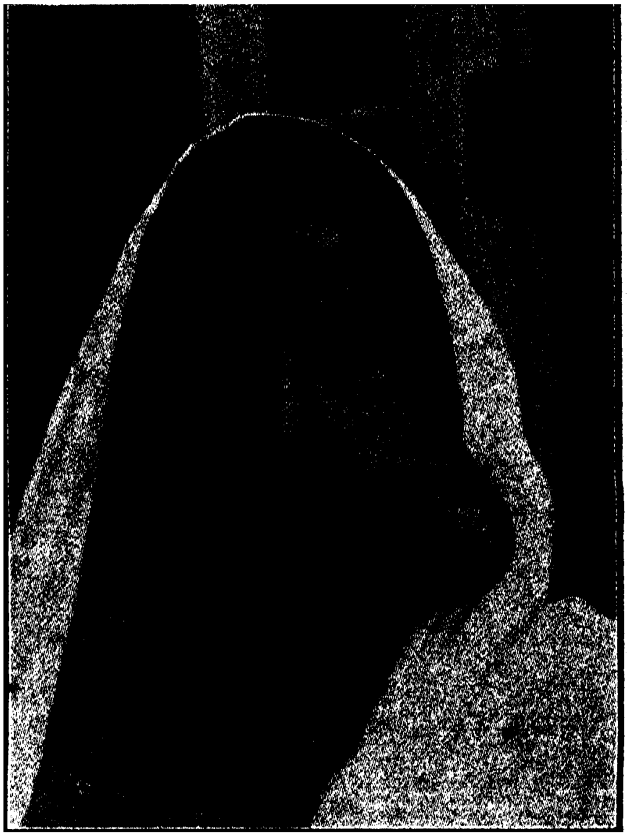


Fig. 213. Sarcoma of the jaw.
(From Dr. P. K. Mitra)

Spindle Celled Sarcoma. May be of two varieties—small and large according to the size of the cells.

Small Spindle Celled Sarcoma. The cells are elongated and spindle shaped. There is very few intercellular material and the vessel wall is defective.

Large Spindle Celled Sarcoma. The cells are elongated and generally arranged in parallel rows in bundles or whorls. The nucleus is vesicular and stains deeply. The cells run in different directions; hence, in examining a section one often meets with all forms of cells according to the plane in which they are cut. Thus the cells cut longitudinally will appear as spindle cells, those cut obliquely will appear as oval cells and those cut transversely will appear as round cells. The intercellular material in this form of sarcoma is usually scanty and very delicate. The blood vessels are wide and very thin-walled.

Seat of Occurrence.—They grow from fascia, aponeurosis, tendons, periosteum, breast, leg, liver, etc.

Mixed Celled Sarcoma. In this form several varieties of cells may occur. Some of them may be oval, some polygonal and some may be even giant cells with several nuclei, or one very big cell containing a deeply stained nucleus. This form of sarcoma generally arises from the internal organs or the lymph glands.

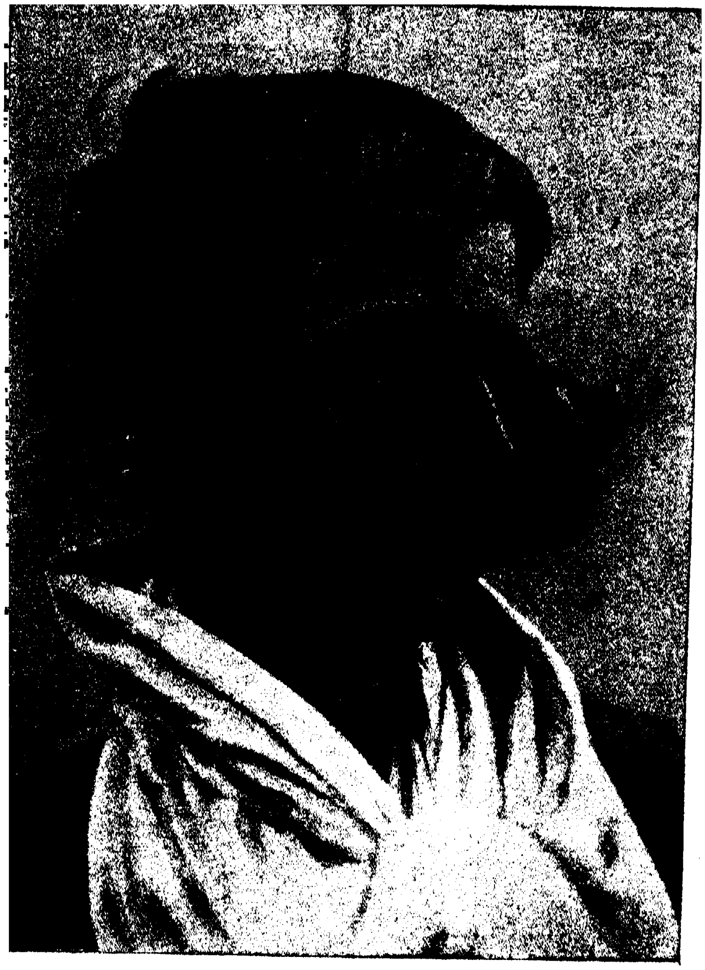


Fig. 214. Sarcoma eye.
(From Calcutta Medical Journal).

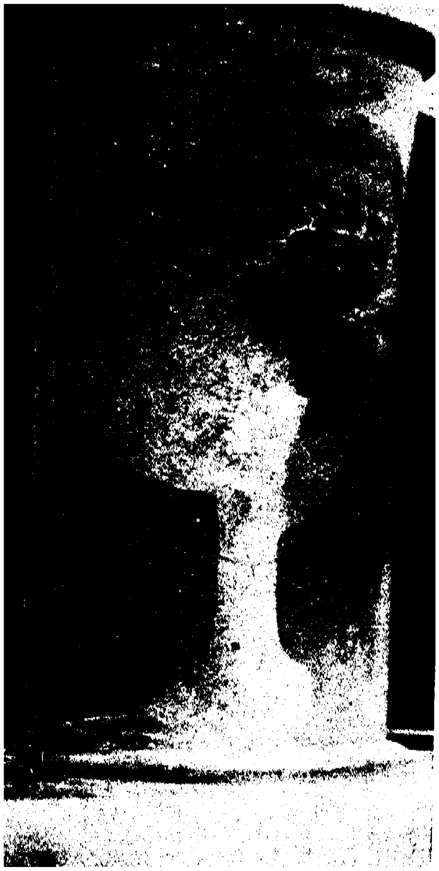


Fig. 215. Osteosarcoma.
(From Pathological Museum Calcutta Medical College).

Alveolar Sarcoma. Alveolar arrangement is the peculiarity of carcinoma and rare in sarcoma. In some cases of sarcoma the cells are arranged in alveoli lined by hands of well developed fibrous tissue stroma and hence these forms are known as alveolar sarcoma. The cells are round or polygonal and generally grow in groups. The existence of this variety of sarcoma is denied by many. They regard this to be a form of endothelioma.

This is differentiated from carcinoma by the following:—

ALVEOLAR SARCOMA.	CARCINOMA.
Stroma present between individual cells.	Stroma present round many cells forming the alveoli.
Wall of blood vessels deficient.	Wall of blood vessels normal.
The alveoli are full of cells.	The cells often shrink from the wall during fixing and staining process.
Examination of several sections shows its origin from connective tissue.	Epithelial origin is found on examination of several sections from different parts of the tumour.

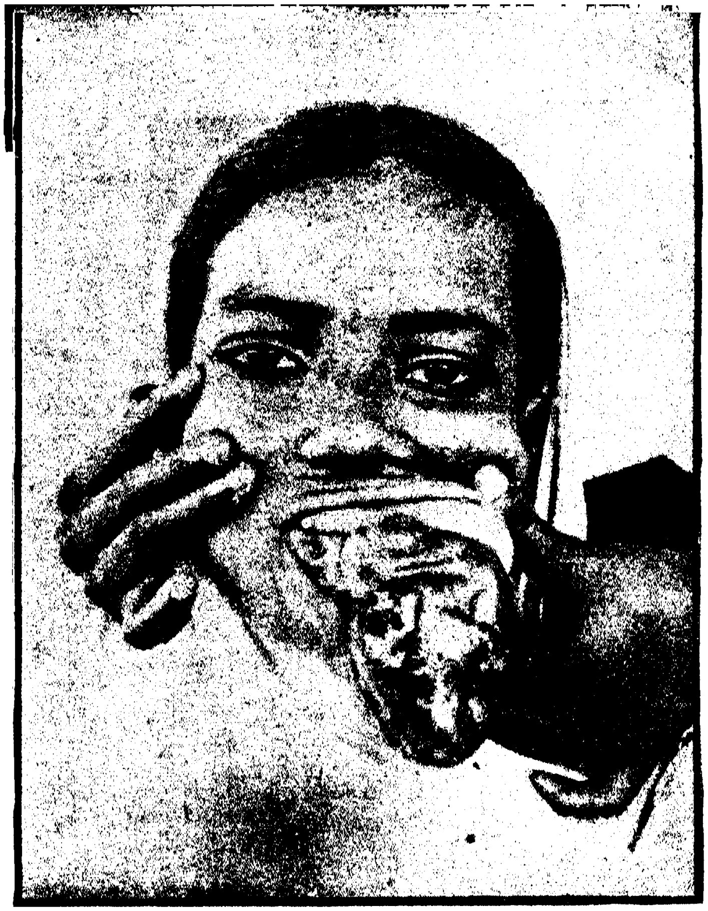


Fig. 216. Malignant epulis.
(From Carmichael Medical College Hospital).

SEAT OF OCCURRENCE.—Skin, bone, lymphatic glands, serous membranes, etc.

Mixed Sarcomata.—These are sarcomatous growths in which the cells of both the embryonic and adult types are present. It is believed that the cells of the affected portion multiply so rapidly that no time is available for the cells to undergo maturation and become adult connective tissue.

Fibrosarcoma.— It is a slow growing, firm tumour and is more cellular than ordinary fibroma. Often it offers great difficulty to differentiate soft fibroma from fibrosarcoma histologically.

Myxosarcoma.—This generally takes place in a structure rich in myxomatous tissue, but may also be a retrogressive process of a pure sarcoma undergoing mucoid degeneration, so common in spindle-celled sarcoma.

Liposarcoma.—This grows from fatty tissue or may be due to fatty degeneration of a true sarcoma. This form is very rare.

Chondrosarcoma.—This grows from a cartilaginous structure. It is a slow growing tumour and is very liable to mucoid degeneration.

Osteosarcoma.—This grows from a bony structure or may also be the result of calcareous infiltration in a chondro—or fibrosarcoma. This grows from the periosteum, endosteum or the bone itself. It shows a ground-substance of spindle-shaped cells with a considerable amount of bone-forming cells, cartilage, osteoid and osseous tissue. Calcification and ossification of the tissues may produce radially arranged spicules and lamellæ of bone. Osteosarcoma produces metastasis in lungs and other distant organs showing atypical cartilage and bone.

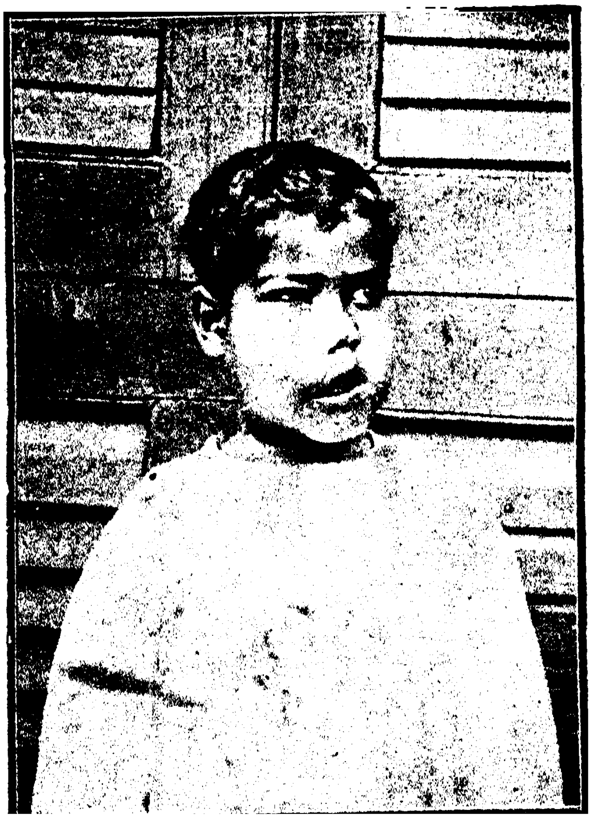


Fig. 217. Multiple sarcoma of both superior maxillæ and right inferior maxilla in a patient aged 4 years.

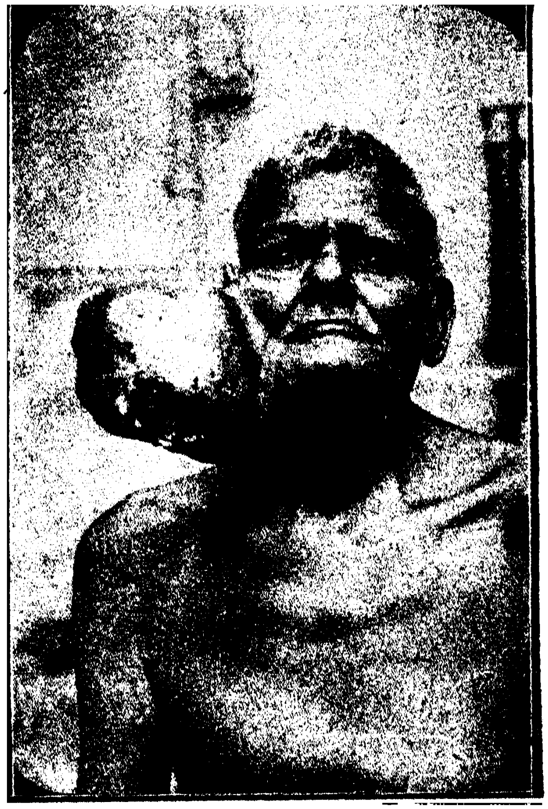


Fig. 218. Malignant Parotid tumour (18 months).
(From Carmichael Medical College Hospital).

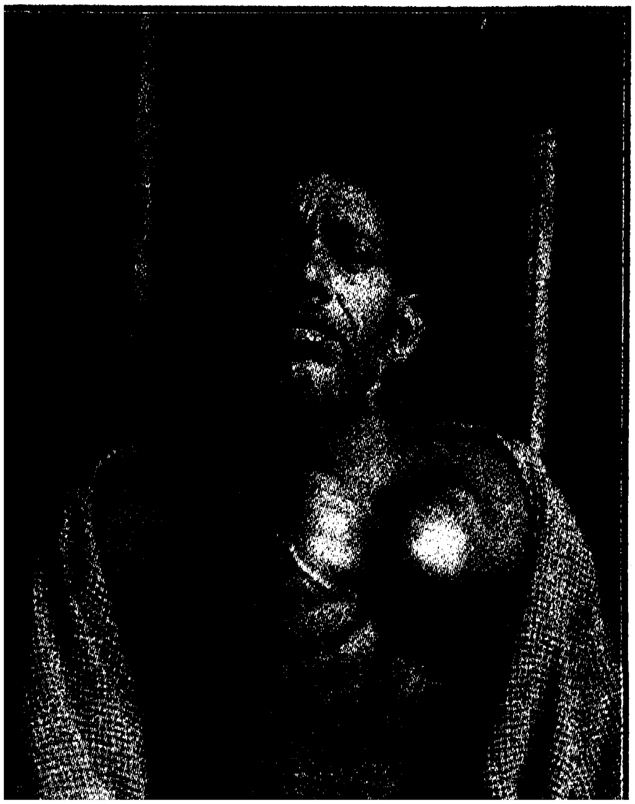


Fig. 219. Mediastinal tumour (malignant). (From Dr. P. K. Mitra)

Myosarcoma.—This is derived from muscular tissue. In case of unstriped muscle tissue it is generally known as leiomyosarcoma and in case of striped muscle fibres rhabdomyosarcoma.

Myelosarcoma or Myeloid Sarcoma or Giant-Cell Sarcoma.—This originates from bone. The so called myeloid sarcoma is rather benign tumour and hence called myeloma. Only when they turn towards malignancy they are called myeloid

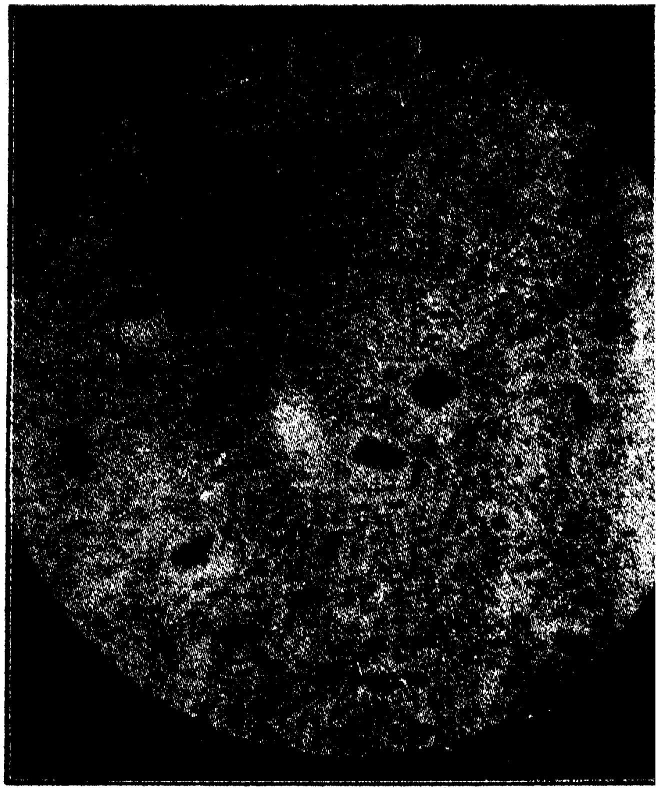


Fig. 220. Giant-celled Sarcoma (High power).

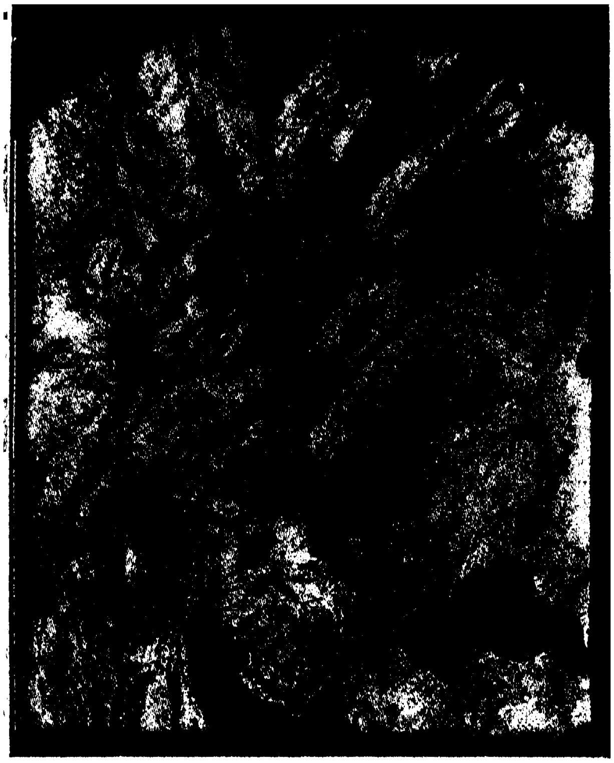


Fig. 221. Osteo-Sarcoma.

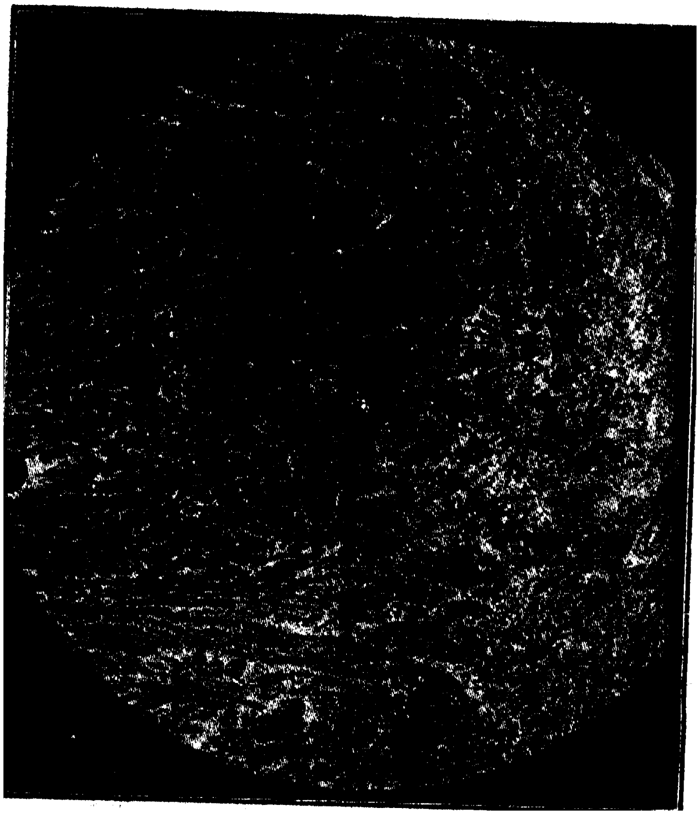


Fig. 222. Malignant melanoma.

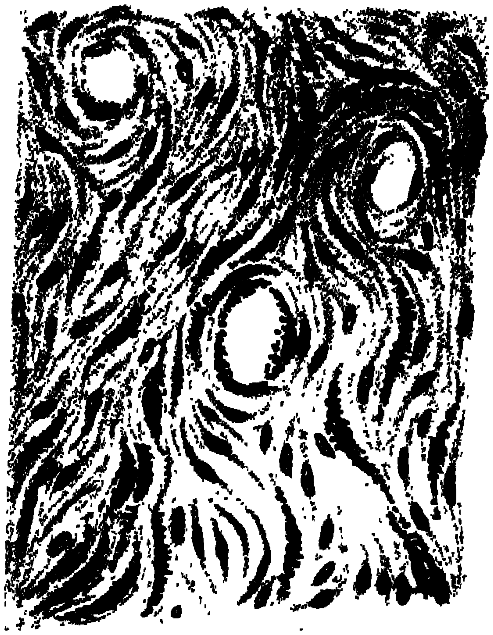


Fig. 223. Melanoma.

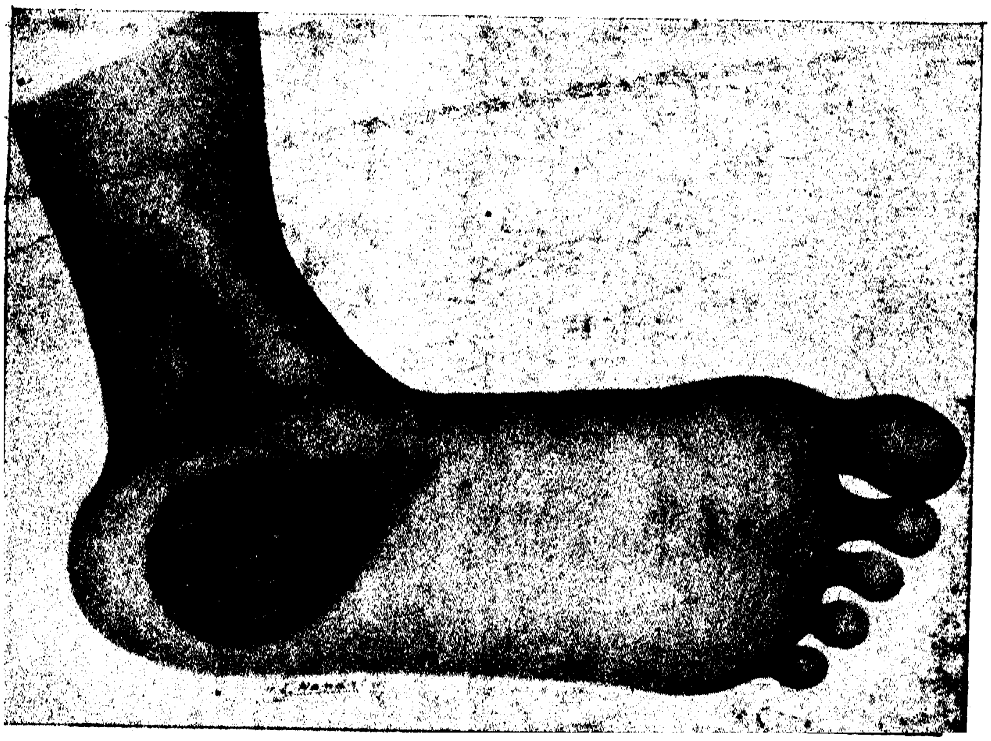


Fig. 224. Melanotic Sarcoma of the foot.
(From Pathological Museum, Calcutta Medical College).

sarcoma. (*See* also myeloma). It is said that the giant cells are identical with the osteoclasts of the bone, which are phagocytic and are of endosteal or periosteal origin. In the breast and other structures, having no connection with bone, there occurs giant-cell sarcoma and so they are also regarded to be of the type of foreign-body giant cells *Epulis,* growing from the alveolar process of the jaw, is the commonest form of giant-cell sarcoma. They form projected masses inside the mouth. Ulceration takes place rather early but they show little tendency to recur after removal or to cause metastasis.

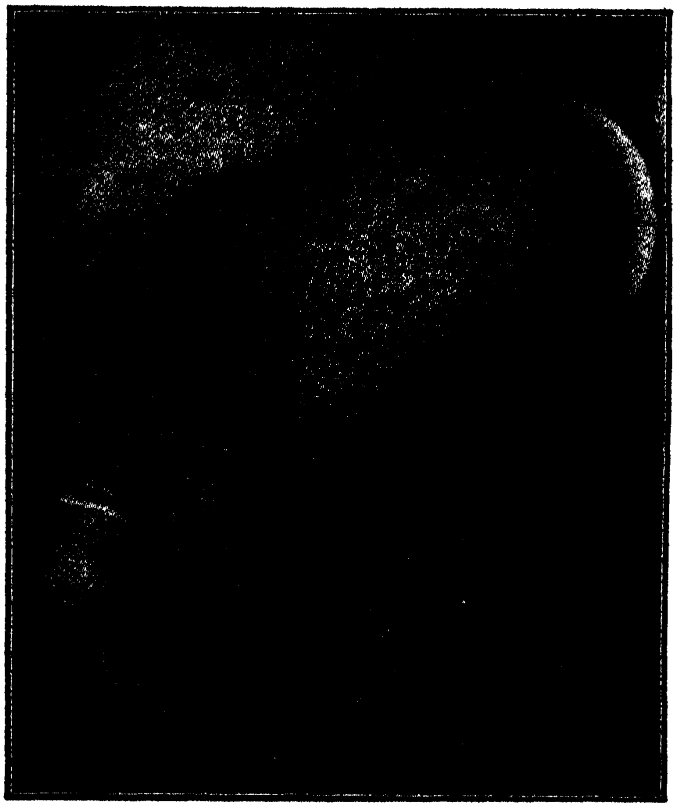


Fig. 225. Tumour of undesended testis. (From Dr. P. K. Mitra).

Fig. 226. Melanotic Sarcoma of the liver. (From Pathological Museum, Cal Med. College)

Melanotic Sarcoma.—This originates from the tissues which are pigmented normally. The pigment-bearing cells or the chromatophores are normally present in the skin and choroid coat of the eye. Those of the skin are generally believed to be of epithelial origin and the malignant tumours originating from the same are called by some writers as the melano-or nævocarcinoma. The malignant growths from the chromatophores of mesoblastic origin are called the melano—, or the melanotic sarcoma.

Whatever may be the source of origin, the melanotic tumours show the same general characters. From this point of view it would be better to use the term **melanoma** for the whole group whether growing from epiblastic or mesoblastic tissue.

SEAT OF OCCURRENCE.—Choroid of the eye, skin, conjunctiva, ovary, the adrenal, etc.

HISTOLOGICALLY.—The cells of melanotic sarcoma are generally of spindle cell types, which are loaded with the pigment. Apart from the pigment, the structure is that of a spindle celled sarcoma. The pigment is melanin and contains sulphur, but no iron.

The secondary growths take place early and, in most instances, before the primary focus can be detected, the patient dies of secondary metastases which are nearly always multiple. It is thus one of the most malignant tumours. The amount of pigment varies a good deal, both in the primary as well as in the secondary deposits.

Gliosarcoma.—This is derived from retina or neuroglial tissue. It consists of cells only, with a few neuroglia fibrils.

ENDOTHELIOMA

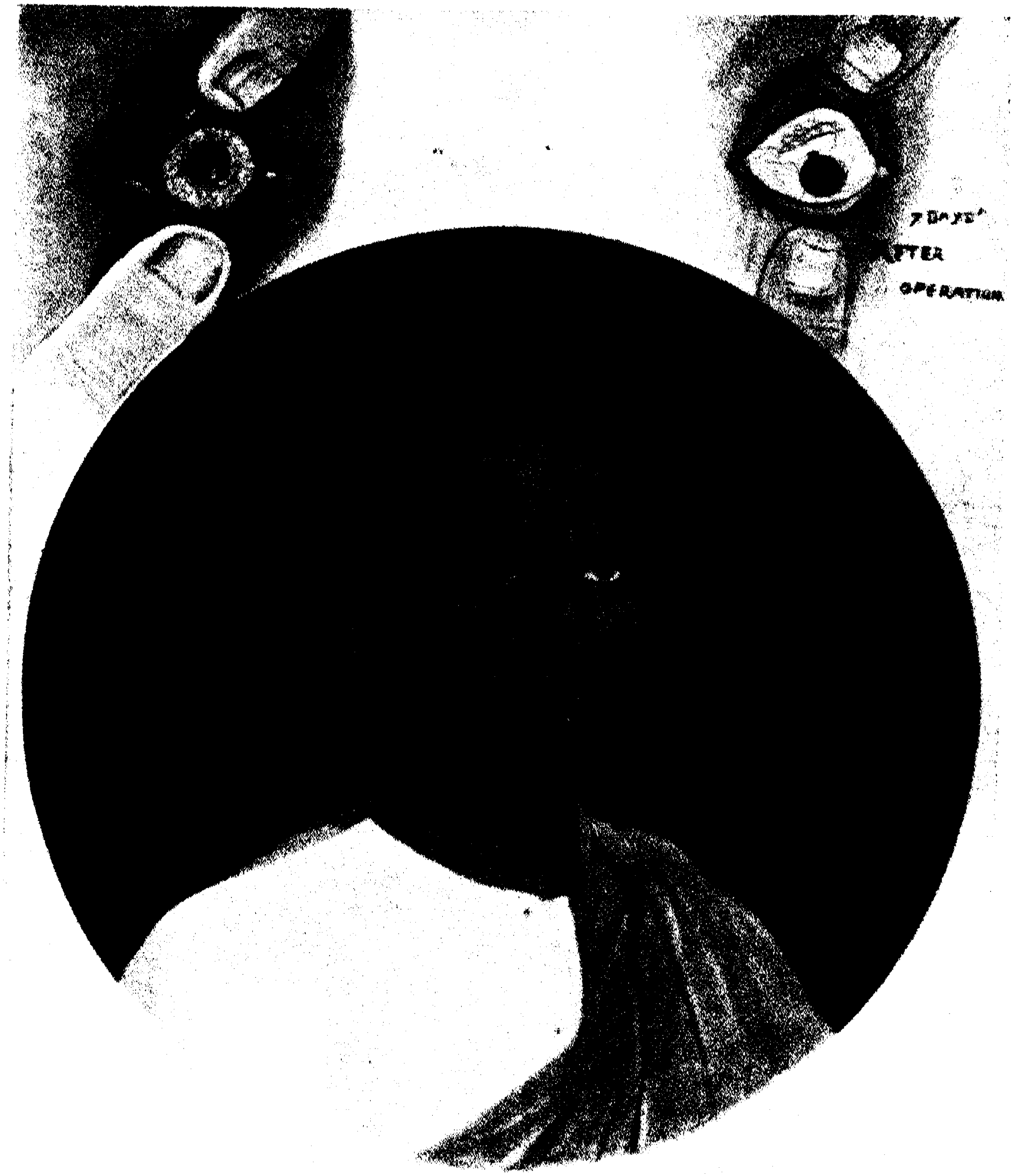
The endothelioma is a tumour, originating from the endothelial lining of blood vessels, lymph vessels and serous cavities.

Seat of occurrence.—It is widely distributed and may occur in every tissue where endothelium is present.

Morbid anatomy.—The growths are single or multiple, nodular or flat, more or less solid in consistency and gradually extending over the whole of the endothelial membrane. In cases of serous membranes, they are thickened and adherent, occasionally obliterating the serous cavity.

Histology.—The endothelial cells are more or less polyhedral, cylindrical or pavement in form. The shape varies widely due to their mutual pressure, when it may assume a spindle shape and may be spheroidal in type, when occurring in œdematous tissue. The cells are composed of well defined cell walls, enclosing clear cytoplasm, with a small vesicular nucleus, showing numerous nucleoli due to mitotic changes. On account of the cells resembling epithelium, the tissue is most apt to be mistaken for epithelioma and in case of spindle cells it is mistaken for a sarcoma. The present day conception about

Plate No. VII.



A case of unilateral glaucoma of the right eye with nævoid patch on the affected side of the face, forehead and brow

From Calcutta Medical Journal.)

Face Page 464.

the nature of endothelioma is extremely imperfect. In obscure cases, where the tumour presents unusual characters, it is very often labelled endothelioma, only to cloak our ignorance. There are divergent views as regards the development of these tumours. The accepted and at the same time the most convenient view is to classify them as follows:—(*a*) Endothelioma originating from the cells lining the blood vessels—Hæmangio-endothelioma or hæmangioma or simply angioma. (*b*) Endothelioma originating from the cells lining the lymph vessels—Lymph-angio-endothelioma or lymphangioma. (*c*) originating from the cells lining the serous membranes,* specially those of the meninges—(*i*) Psammoma, (*ii*) Cylindroma (*iii*) Cholesteatoma. (*d*) Endothelioma originating from other structures and commonly mistaken for carcinoma, *e.g.*, mixed parotid tumours and similar tumours in connection with the breast, uterus, etc. Mixed parotid tumours with presence of cartilages are included under teratoma.

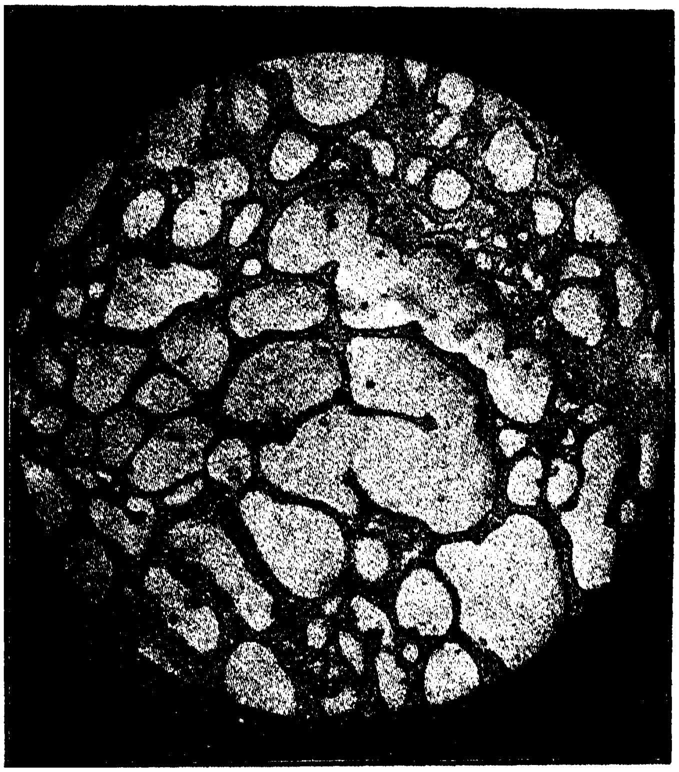


Fig. 227. Cavernous angioma.

ANGIOMA

The angioma as mentioned above is a tumour composed of newly formed vessels. They are classified as follows:—(*a*) Growing from blood vessels—hæmangioma. (*i*) Capillary hæmangioma. (*ii*) Cavernous hæmangioma. (*b*) Growing from lymph vessels—lymphangioma.

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* Of the serous membranes, the lining of the cerebro-spinal spaces, are accepted to be lined by endothelium, whereas, according to the cœlomic theory, the lining-cells of the pleura, peritoneum, pericardium, etc., are of epiblastic (hypoblastic and epiblastic) in origin; and the tumour, originating from these serosa cells, are regarded as epithelial tumours.

Hæmangioma

Hæmangioma must be distinguished from conditions in which there is unusual blood supply, due to overgrowth of the vessels. They are as follows:—(*a*) Varicose veins in the limbs, spermatic cords, etc. In these conditions there is an extreme development of the blood vessels and so these do not constitute neoplasms. (*b*) Granulation tissue.—In this condition there is extensive proliferation of newly formed blood capillaries. There is complete absence of the characters of a tumour in the granulation tissue.

Capillary Angioma

Synonyms.—Hæmangioma simplex, vascular nævus.

In this condition the blood vessels are smaller in size and very great in number. They are usually congenital.

Seat of occurrence.—They occur in the subcutaneous tissue, mainly of the face, lips and trunk. They also occur in the muscles of the limbs.

Morbid anatomy.—There may be definite enlargement and occasionally a tumour formation of the subcutaneous tissue. The birth-marks are not raised above the surface, the epidermis becoming thin and delicate. The part looks red, pink or black and there may be turgescence and impairment of function of the part.

Histology.—The tumour does not affect the whole blood vessel but is formed from an isolated portion of it. The blood vessels are hypertrophied with formation of a large number of capillaries lined by hypertrophic endothelium. The supporting connective tissue is also hypertrophied. There may be slight or no connection between the newly formed blood vessels and the original normal vessels of the part.

Cavernous Angioma

Seat of occurrence.—Occurs in nearly all tissues and organs, especially the skin, subcutaneous tissue, liver, breast, lungs, tongue, lips, eyelids, conjunctiva, parotid gland, ovary, uterus, brain, etc.

Morbid anatomy.—They are circumscribed or diffuse, slightly elevated, pigmented growths. They are usually small, but may attain large dimensions. They are slowly-growing and benign in nature.

They often recur after incomplete removal. Cavernous angioma of the liver, as a rule, produces no symptoms, and is found only at autopsy. It may be single or multiple.

Histology.—The vascular channels are widely dilated with very little, connective tissue stroma between. The vessels are lined by endothelium and they are in constant anastomosis with one another. Ribbert has proved by injections that the capillaries of an angioma have no communication with those of the surrounding tissue.

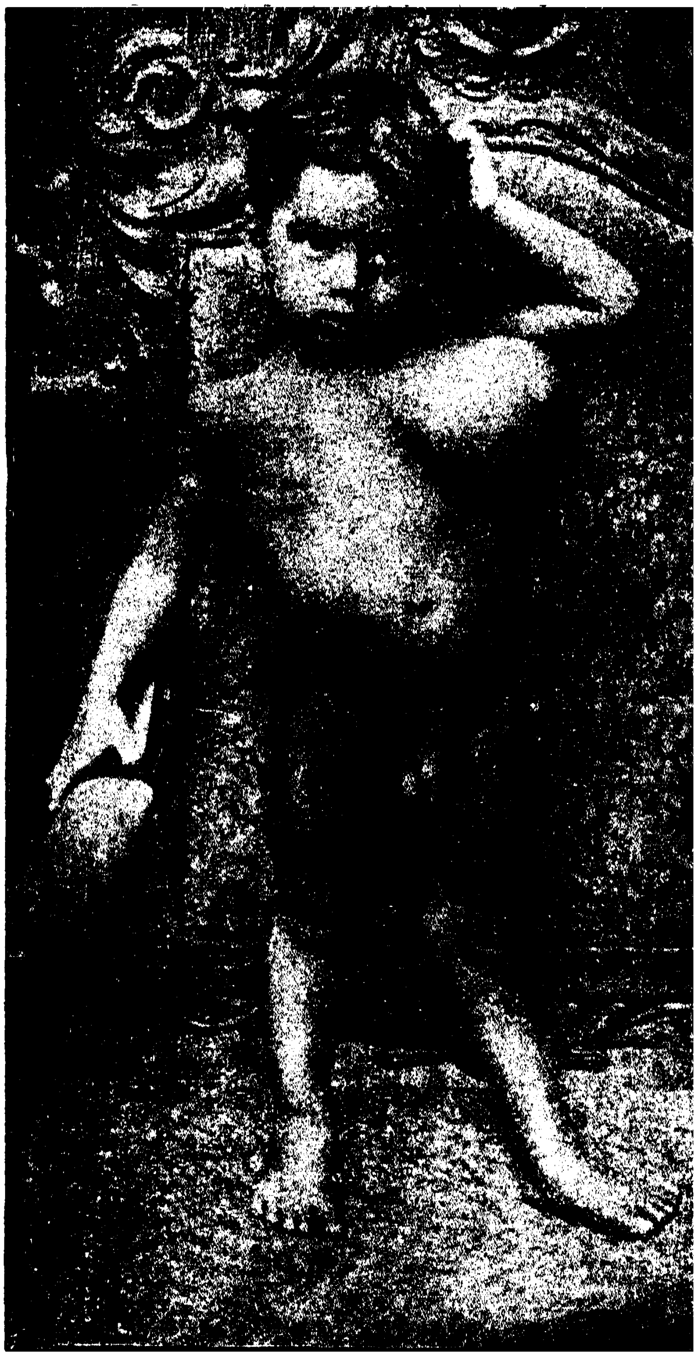

Fig. 228. Cystic hygroma.
(From Carmichael Medical College Hospital).

Lymphangioma

Lymphangioma is a tumour composed of lymph vessels.

Seat of occurence.—They occur usually in the skin, subcutaneous tissue, muscles of the neck, tongue, lips, orbit, and in the mediastinal and retroperitoneal regions.

Morbid anatomy.—They are slowly growing tumours single or multiple. Some of them contain blood vessels as well, forming what is known as hæmolymphangioma.

Histology.—It consists of well formed lymph vessels lined by endothelium and supported by fibrous tissue matrix. The lymph vessels and spaces anastomose freely, but, as in the hæmangioma, the lymph channels are not in communication with the adjacent lymphatics.

Varieties.—*Simple Lymphangioma.*—This mainly affects the skin and is composed of moderately dilated vessels. They are commonly found in filarial elephantiasis and lymphscrotum, which consist of dilated lymph vessels (lymphangiectasis) with œdema due to obliteration of lymph channels by the adult filaria. Secondary infection and subsequent inflammation form a great part in the production of elephantiasis, for which reason, some authorities do not include this condition under the tumours. Simple lymphangioma occurs in the skin of the face, neck and other parts, as small or large flat prominences.

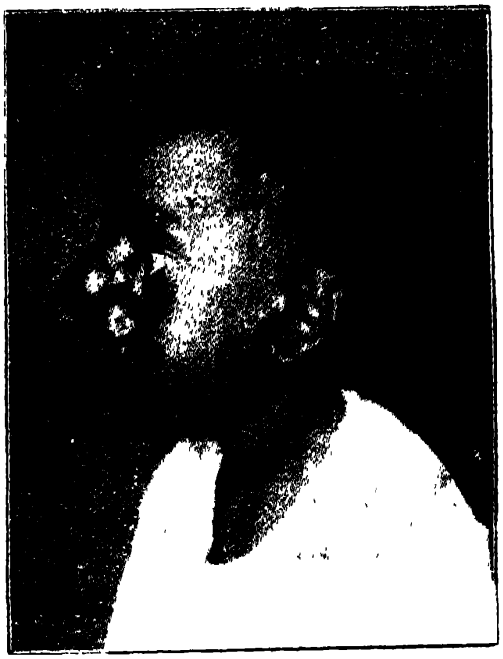

Fig. 229. Lymphangioma.
(From Dr. P. K. Mitra)

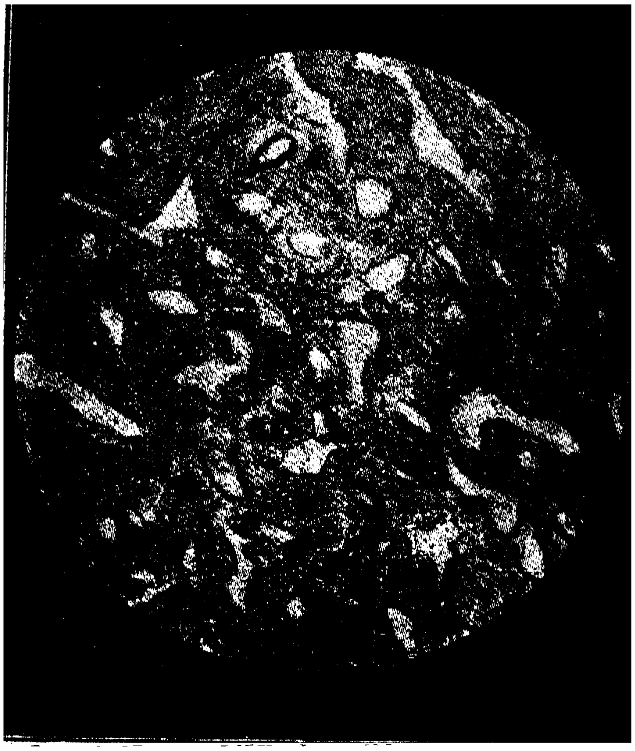

Fig. 230. Lymphangioma.

Cavernous Lymphangioma.—In this condition, the vessels are greatly distended and contain lymph which is readily coagulable and is often intermixed with blood.

Chylangioma.—This usually occurs in the mesentery and is a form of cavernous lymphangioma containing chyle due to obstruction of the lacteals. Chylangioma must be distinguished from simple dilatation of the chyle-ducts, *Cystic Hygroma.*—This is a form of lymphangioma, usually occurring in the submaxillary region, neck, axilla and the sacral region. In the last mentioned place, it develops from some defects in the spinal canal. It is generally due to disturbances of development.

Psammoma

This is an endotheliomatous tumour, originating from the membranes of the brain and spinal cord. It is a benign, small, hard, circumscribed and nodular growth which gives a gritty feel when incised with a knife.

Histologically it presents (*a*) Abundance of spindle cells. (*b*) Hyaline degeneration of areolar tissue. (*c*) Calcareous infiltration of endothelial cells.

Cylindroma

This is an endotheliomatous tumour originating from the meninges, peritoneum, jaw, etc. It is very vascular and shows hyaline degeneration of the walls of the blood vessels.

Cholesteatoma

This tumour originates from the pia mater and other parts of the brain and middle ear with the formation of large number of rounded bodies in the areolar tissue resembling "epithelial pearls" of squamous epithelioma. Cholesterin and even hairs are occasionally present in them.

Endothelioma Originating from other Situations

These present a difficult, confusing and anomalous condition. The arrangement of the cells and fibrous tissue occasionally resembles carcinoma from which it is often very difficult to differentiate.

Mixed Parotid Tumour

This may be taken as a type of this form of endothelioma. It is a more or less circumscribed, firm, slowly growing, slowly metastatic, lobulated tumour with slight degree of malignancy. It often recurs after removal which is due mainly to imperfect removal of the whole mass.

Histologically.—It consists of cubical or flattened group of cells often in masses and surrounded by a fibrous stroma. The group of cells often shows a tendency to enclose distinct lumen which often contain blood. It has a marked tendency to undergo mucoid degeneration. These myxomatous cells often resemble cartilage cells. In some instances true cartilage is often found. Mixed parotid tumours with presence of true cartilage cells are generally grouped under teratoma.

Perithelioma

Perithelioma is a sub-variety of endothelioma originating from the endothelium of perivascular lymphatics. These tumours usually grow rapidly. They are vascular and contain large cells, in several layers, arranged round the blood vessels. They generally involve the pia mater, ependyma, etc., and are classified as midway between sarcoma and endothelioma.

NEUROMA

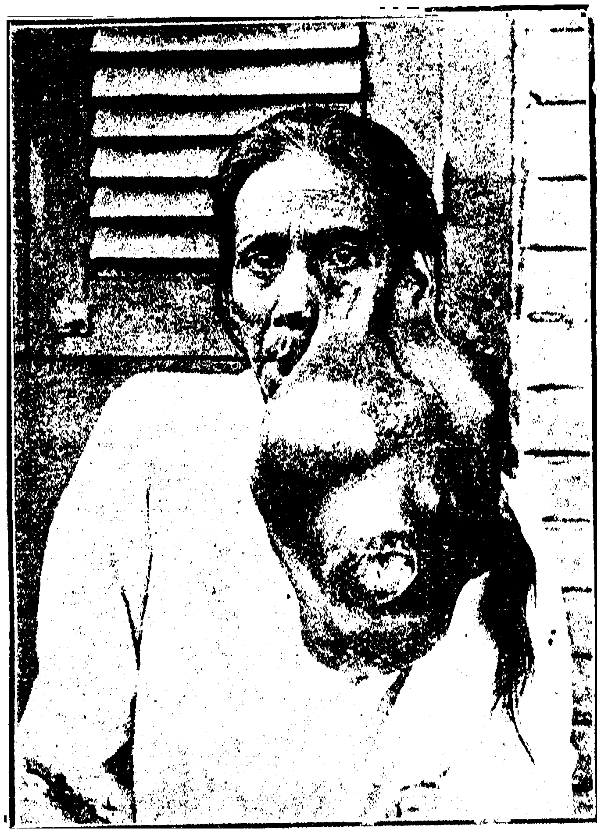

Fig. 231. Mixed parotid tumour

Neuroma is a tumour composed of nerve cells and nerve fibres. This often [illegible] *ma* when the tumour is composed mainly of cells, and *myelinic neuroma*—when this is composed of nerve fibres mainly.

Ganglionic Neuroma

Seat of occurrence.—It occurs throughout the central nervous system and it is very frequent in connection with the sympathetic nervous system.

Morbid anatomy.—Neuromata are small or large, single, or multiple, nodular growths, growing chiefly in connection with the nerve trunks and plexuses of the sympathetic system. They are found in the regions of the gastrointestinal tract, adrenals, retroperitoneal space and subdural spaces of the central nervous system.

Histology.—In structure they are composed of numerous ganglionic cells with bundles of nerve fibres and supported by connective tissue and blood vessels. The ganglion cells look like large round cells and contain little chromatin substance. The nerve fibres are either medullated or non-medullated. In section ganglionic neuroma often resembles a fibroma originating from nerve trunks.

Neuro-Fibroma.—(*Myelin neuroma*).—These are tumours growing from nerve trunks, and may be either true neuroma or false neuroma.

True Neuroma.—According to Virchow, it consists of masses of long, straight, branching and anastomosing nerve fibres enclosing numerous oval nuclei. Myelin sheath may or may not be present.

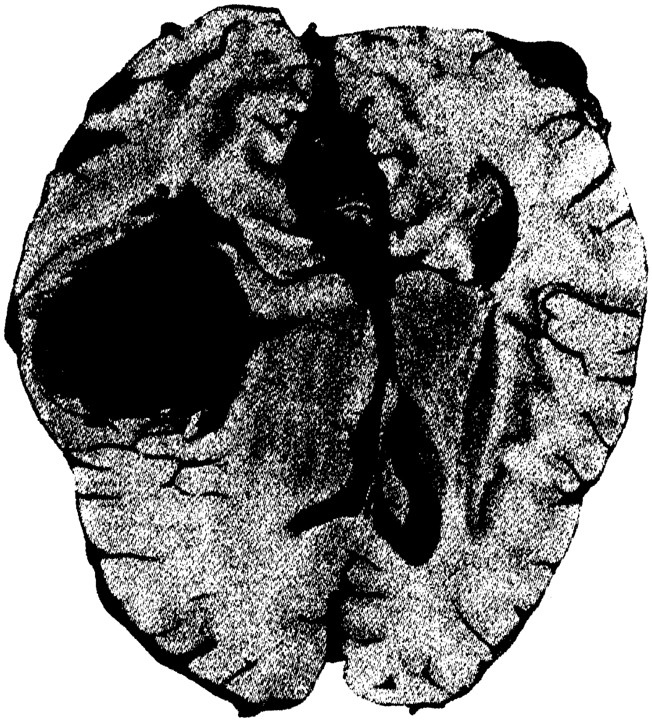

Fig. 232. Neurofibroma of the brain with hæmorrhage.

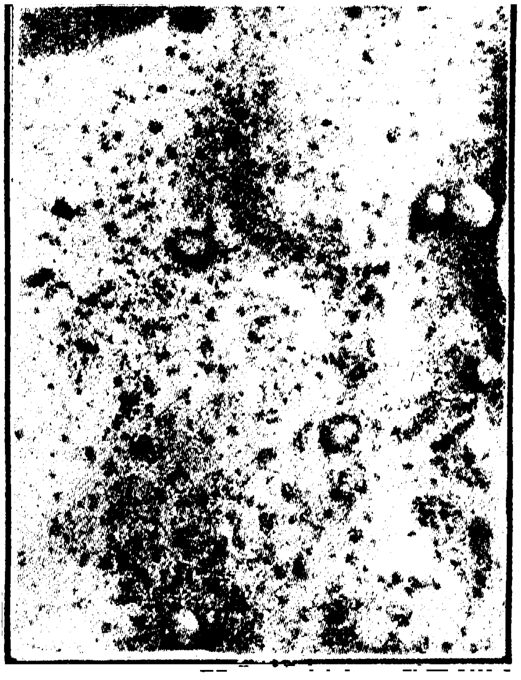

Fig. 233. Neurofibroma of the skin.

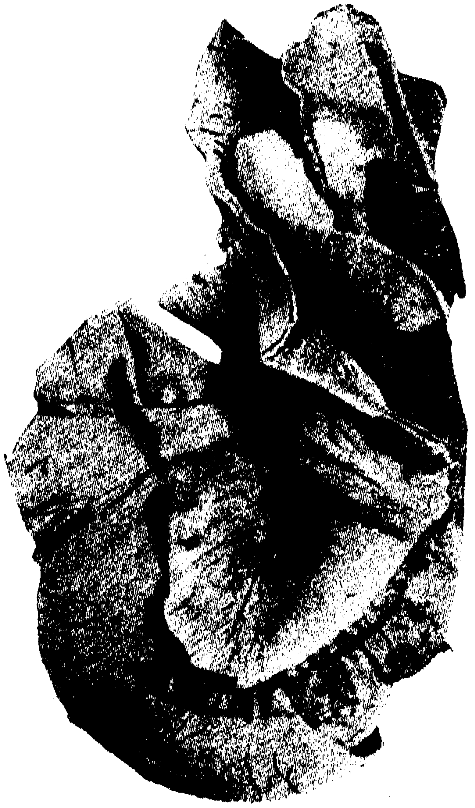

Fig. 234. Neurofibroma of the intestine causing intussusception.

False Neuroma.—These are derived from the connective tissue of the nerve trunks, which proliferate to a considerable extent and lead to the atrophy of the nerve fibres by pressure. They form multiple, small, painful growths and are generally described as multiple neurofibroma of von Recklinghausen.

Traumatic Neuroma.—These are commonly found in amputation stumps or in cases where the peripheral nerves are severely injured. The connective tissue of the injured nerve trunks proliferates, resulting in the thickening of the part, into which the nerve fibres force their way and become elongated and form twisted bundles by being coiled up in the surrounding tissue, with formation of tumour-like masses. The medullary sheath also proliferates and ensheaths the newly formed axis cylinder.

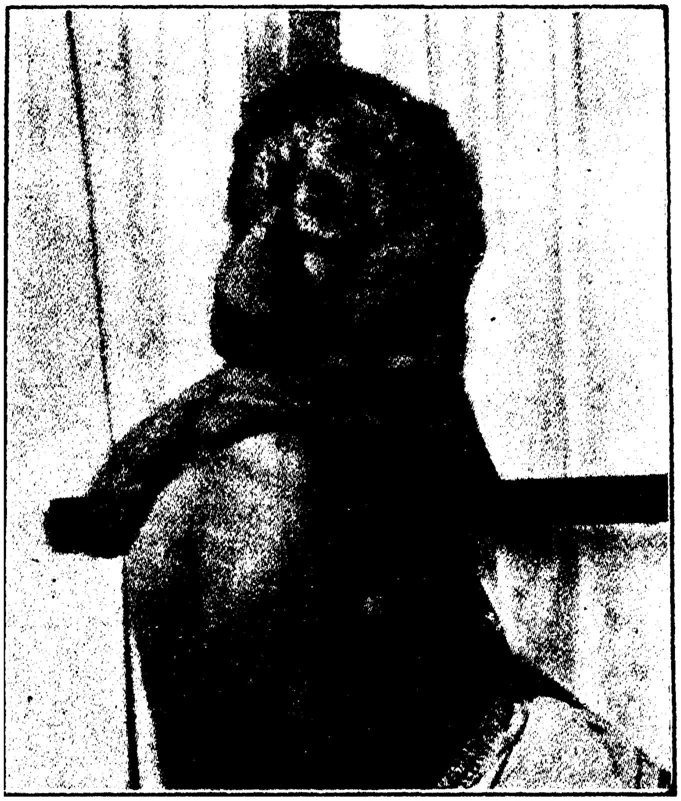

Fig. 235. Von Recklinghausen's disease. (From Dr. P. K. Mitra).

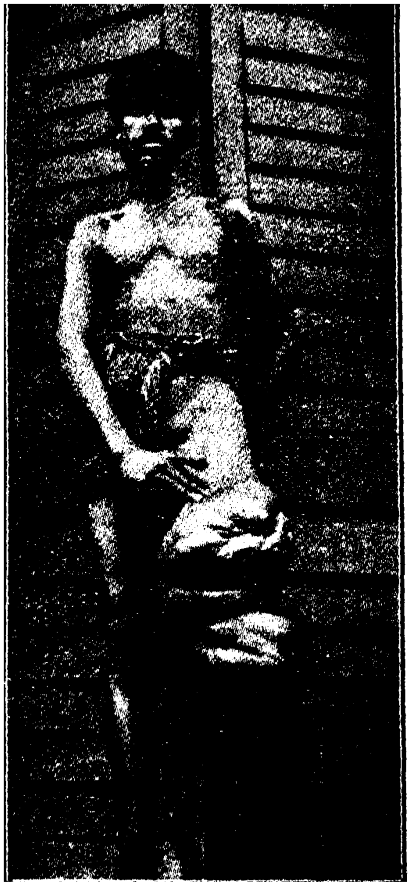

Fig. 236. Von Recklinghausen's disease. (From Dr. P. K. Mitra).

Cirsoid Neuroma.—This consists of thickened and elongated nerve trunks. This is supposed to be intermediate between true neuroma and neurofibroma.

Ganglioneuroma.—This consists of non-medullated nerve-fibres and imperfectly developed ganglion-cells. This is supposed to take its origin from the sympathetic system and is usually found in retroperitoneal, retrocervical or pelvic regions.

GLIOMA

Glioma is a tumour originating from neuroglia or the supporting connective tissue of the nervous system. Neuroglia is epiblastic in origin.

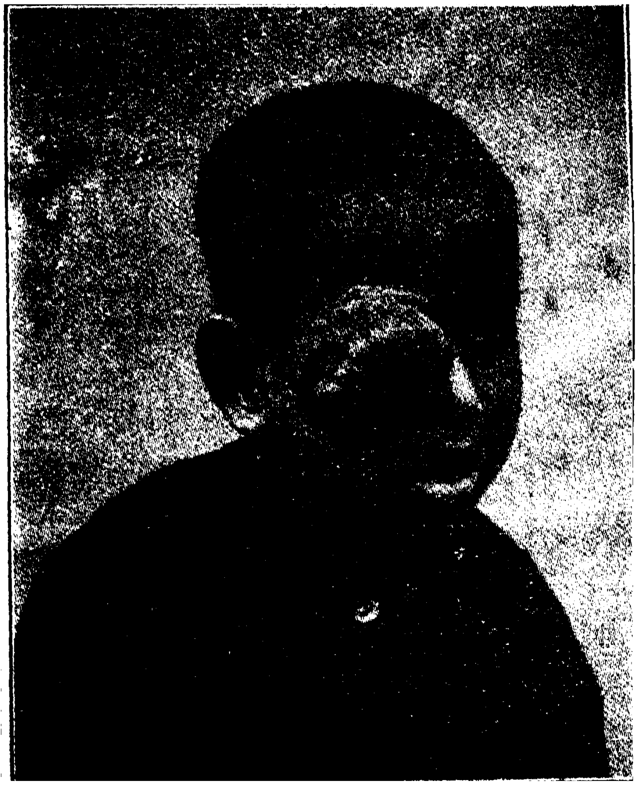

Fig. 237. Glioma of the eye. (From Dr. Charubrata Roy's collection).

Seat of occurrence. It is found in the central nervous system and its outgrowths. It is fairly common in the eye.

Morbid anatomy. It is usually a soft tumour but may be fairly hard in case of growths, containing fibrous tissue in them. It is usually small, but may grow to a considerable size. The margins are, as a rule, not sharply defined. It is not encapsulated. Degenerative changes and hæmorrhages are common on account of vascular disturbances.

Histology. The tissue consists of a large number of glial cells and a few fibrils. The cells are small, rounded, with deeply staining nuclei and occasionally possess long branched processes. The fibrils are very fine and are often lost. Occasionally the cells possess considerable amount of cytoplasm, with numerous long filamentous processes, running in all directions, for which they are called spider cells or astrocytes. The histological picture alters to a great extent, due to the presence of hæmorrhages, necrosis and other degenerative changes of the tumour tissue. In rare occasions the degenerative area may be seen to have been replaced by fluid, forming a cyst.

Disposition. They are slow-growing benign tumours, but may prove fatal on account of their peculiar position. In case of affection of the retina, the growths recur after excision and show great extensions in all directions, *e.g.*, into the cranial cavity, to the other eye, towards the face, etc. These growths give rise to no metastasis and from that point of view, glioma may be regarded to be benign in nature although the growths are destructive to a considerable extent, by means of infiltration of the vital structures. They are very liable to hæmorrhages, with consequent apoplectiform attacks. Serious symptoms are common, due to pressure on the central nervous system.

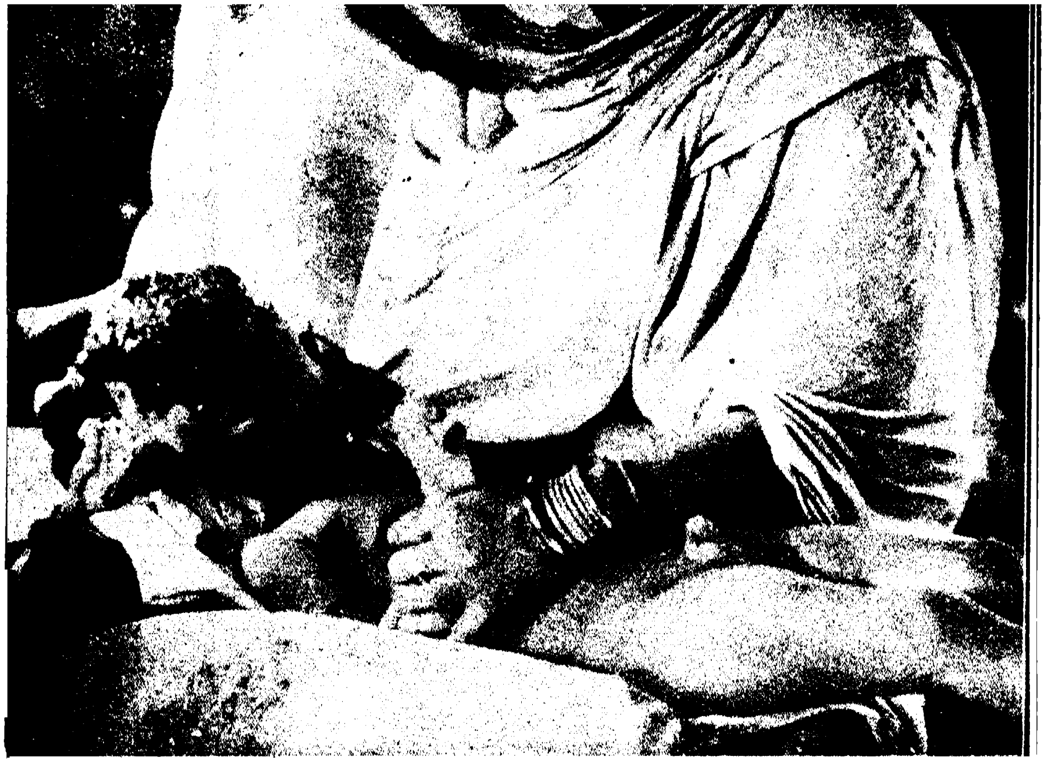

Fig. 238. Glioma of the eyeball. (From the Calcutta Medical Journal).

CHAPTER XXIX

EPITHELIAL TUMOURS

General consideration.—Epithelial cells are found (1) lining the outer surface of the skin, mucous membranes of the channels of the body, *e.g.*, the alimentary, respiratory, urinary, genital and other tracts, and also the ducts of the secretory glands, and (2) in solid masses in epithelial secretory glands. The epithelium is provided with no intercellular material. the cells being placed in close contact with each other. It is nourished, not by the blood, but by the lymphatics. The epithelial cells are separated from the neighbouring structures by connective tissue, called stroma, which form its supporting structure and, as the epithelial cells themselves are not supported by any intercellular material, the stroma is essential for their support and in case of proliferation of the epithelial cells the stroma must also proliferate simultaneously although secondarily. This is the reason why, in all epithelial tumours, proliferation of the connective tissue takes place side by side.

Classification.—This might be grouped according to the distribution of the epithelium.

1. Benign tumours growing from the cells lining the skin, mucous membranes, ducts, etc.—*Papilloma*--the epithelial cell grows and surrounds the stroma.
2. Benign tumours growing from the secretory glands—*Adenoma*—the epithelial cells grow in masses and are surrounded by the stroma.
3. Mixed forms in which the adenomatous growth forms cysts and the epithelium grows into a papillomatous type—*Papilliferous Cystadenoma.*
4. Malignant tumours growing from the skin—*Squamous Celled Carcinoma or Epidermoid Carcinoma.*
5. Malignant tumours growing from the epithelium lining the mucous membranes and ducts—*Columnar Celled Carcinoma, Malignant Adenoma or Adenocarcinoma.*
6. Malignant tumours growing from the secretory glands—*Glandular Celled Carcinoma.*

PAPILLOMA

A papilloma is a benign epithelial tumour, growing from the cells lining the skin, mucous membranes and cysts, and composed of cauliflower-like growths of epithelium, with a stroma carrying blood vessels into each prolongation.

Varieties.—According to their origin papilloma is divided into:—(1) Cutaneous papilloma, or hard papilloma, verruca or wart. (2) Mucous papilloma or soft papilloma or villous papilloma. (3) Intracystic papilloma commonly found in cysts of ovary and breast.

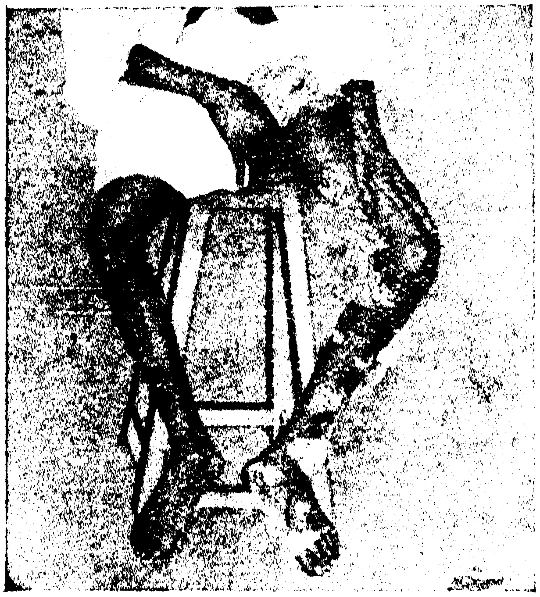

Fig. 239. Multiple papilloma. (From the Pathological Museum, Cal. Med. College)

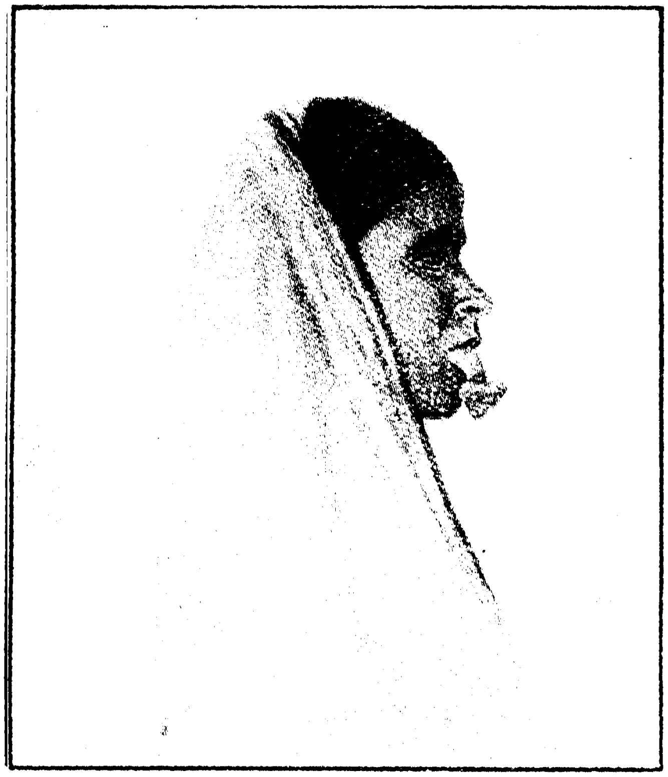

Fig. 240. Papilloma of the tongue.

(1) *Cutaneous Papilloma or wart.*—This, as the term implies, is a small hard tumour originating from the skin generally of the hand, face, and the genital region. It is usually sessile and multiple. Some of these tumours are pigmented and often bear long hairs, the so-called, papillary nævus. In some cases the keratinisation is excessive resulting in the formation of smaller or bigger horns. On account of its position, it is exposed to injuries and so they are frequently ulcerated and inflamed.

In syphilitic conditions the papilloma grows in moist places, *e.g.*, the anus and the genitals and is known as a *condyloma.* In this condition the superficial cells rapidly degenerate.

(2) *Mucous Papilloma.*—Unlike the former variety, this is extremely soft and presents a cauliflower-like villous growth, with long thin processes attached round a central stalk. As they originate from the mucous membrane they cause obstruction to the lumen of the channel if the size is considerable. The neighbouring structures suffer a good deal from the pressure exerted by the tumour.

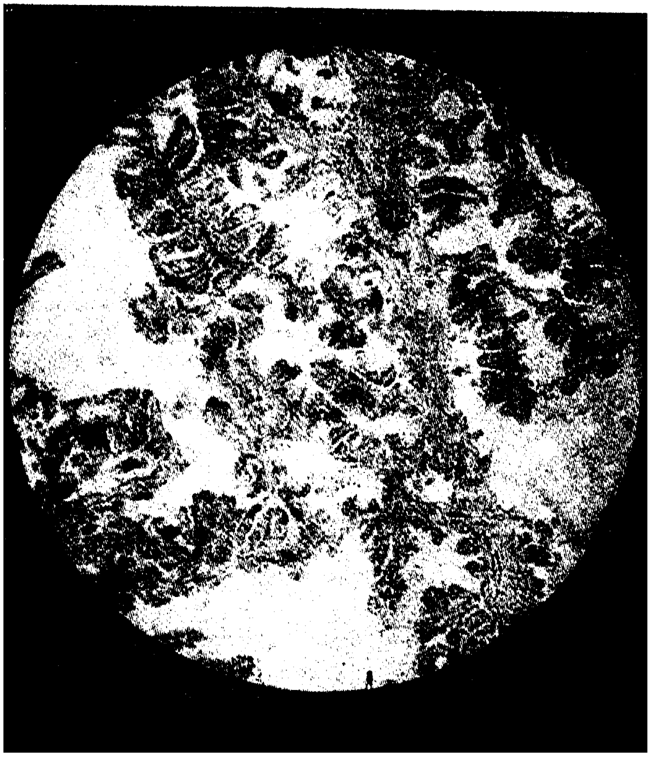

Fig. 241. Papilliferous cystadenoma.

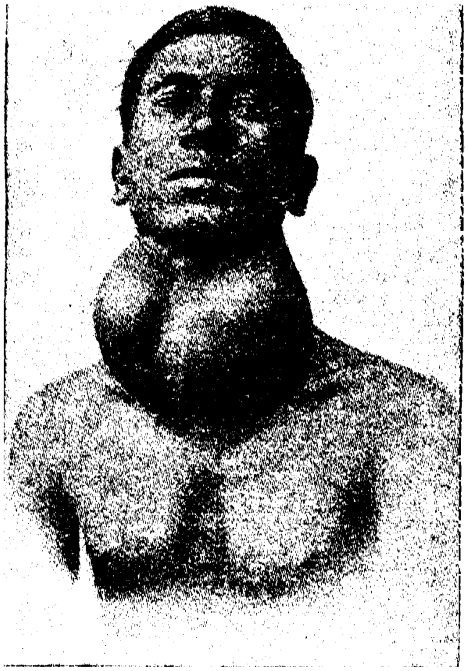

Fig. 242. Adenoma thyroid.

According to the type of cell lining the mucous membranes, mucous papilloma may be—(*a*) *squamous cell mucous papilloma*—originating from the bladder, etc., (*b*) *columnar cell mucous papilloma*—originating from the intestines.

(3) *Intracystic Papilloma.*—These are rare growths originating from the ovary and the breast.

Histology.—The papilloma consists of one, two or more layers of cell, arranged round a central stalk of connective tissue. The vessels are found in the central stalk. The basement membrane is intact. In cases of the cutaneous papilloma, the epithelium multiplies to a considerable extent, so as to form heaps of cells without any corresponding proliferation of the stroma, but still preserving the normal relation of the cells to the basement membrane intact. In rare occasions, the keratosis of the compact epithelial cells takes place in the form of concentric rings, and are known as " epithelial pearls " or " cell nests."

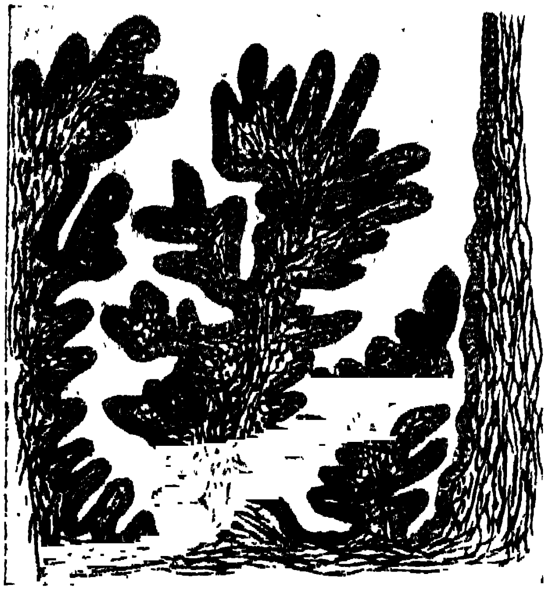

Fig. 243. Papilliferous cystadenoma.

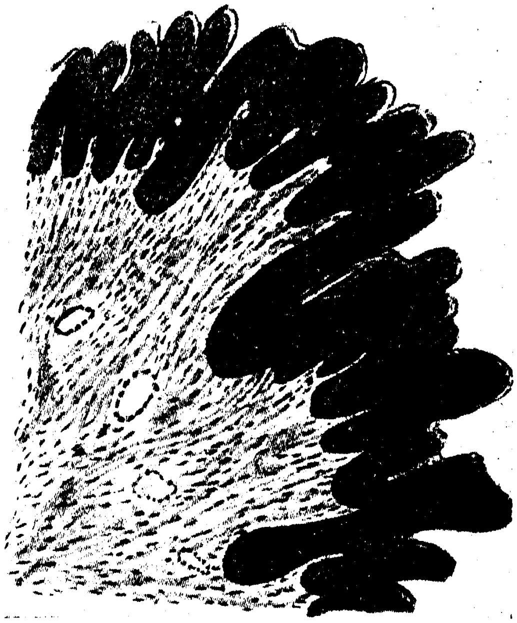

Fig. 244. Papilloma.

ADENOMA

An adenoma is an epithelial tumour with a gland like structure but performing no function of a gland.

Seat of occurrence.—Any epithelial gland may be the site. They are commonly found in the breast, ovary, thyroid, adrenal, liver, kidney, salivary, sweat and other glands, mucous glands of the digestive tract, prostate, hypophysis, etc. The commonest adenomatous growths are found in the breast and the ovary.

Morbid anatomy.—The adenoma is a round, more or less irregular, sessile or pedunculated, well circumscribed and encapsulated growth. It is a benign tumour and grows slowly.

Histology.—The structure closely resembles that of an epithelial gland and consists of acini, ducts and the vascular stroma. Although they are provided with ducts the proper outlet is most deficient and often absent. The character of the cells depends mainly on their origin. Thus the cells are found in masses, when the tumour is originating from a solid glandular structure, such as the liver or a sebaceous gland. They vary in shape according to their origin. They may be columnar or cubical in type. The cells may be one, two or more layers deep, resting upon the vascular fibrous stroma with the basement membrane infact. There is a marked tendency to mucoid degeneration. They occasionally produce the normal secretion of the glandular structure affected *e.g.*, colloid material in case of the thyroid, bile in case of the liver, etc.

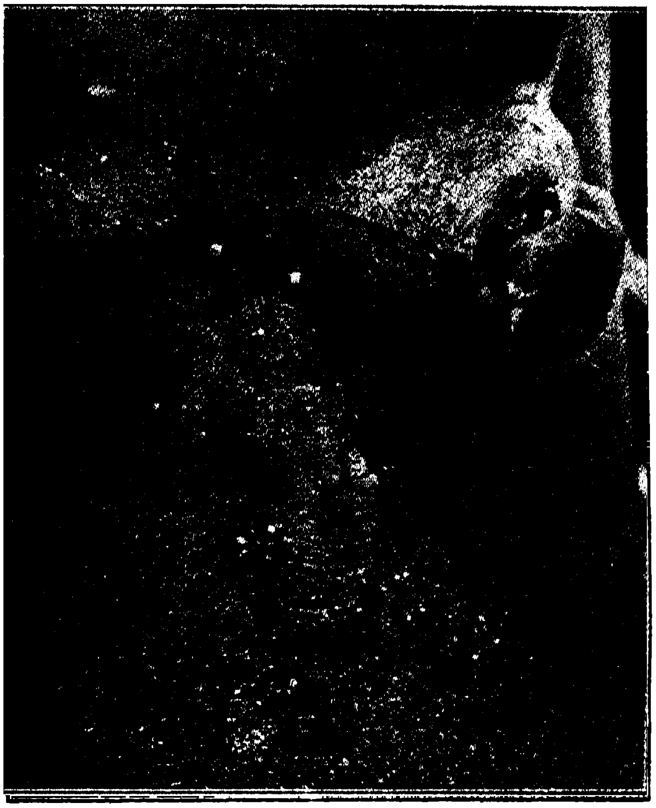

Fig. 245. Horn (From Dr. P K. Mitra).

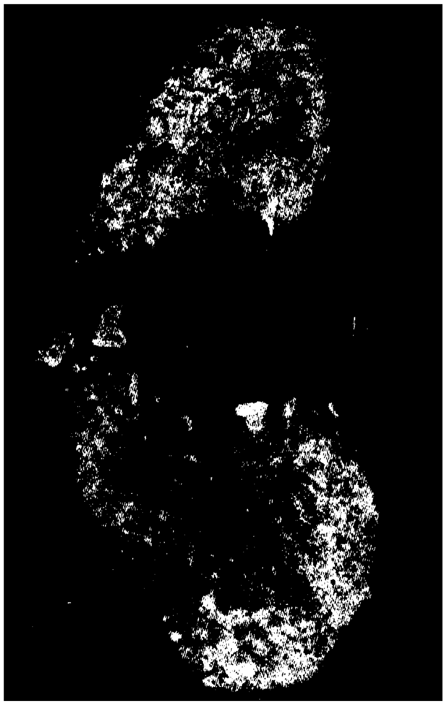

Fig. 246. Total frozen section of Adenoma thyroid The structures between the thyroid glands are.—the trachea in the middle with œsophagus and lymph nodes on each side

Varieties.—This depends on the arrangement of the epithelial cells and their relation with the stroma. *Simple Adenoma.*—When the proportion of cells and the stroma corresponds with that of a normal gland. *Fibro-Adenoma or Adenofibroma.*—When the hyperplasia of the fibrous tissue is excessive. *Alveolar or Racemose Adenoma.*—When the alveolar elements are predominating. *Canalicular or Tubular Adenoma.*—When the canalicular element is predominating. *Cystadenoma.*—When the acini are distended with the retained secretions with formation of cyst-like structures. *Papillary Cystadenoma or Adenocystoma Papilliferum.*—When the cells lining the cysts grow in the lumen as papillary projections.

CARCINOMA

General characters.—The carcinomata or cancers are malignant epithelial tumours. They are essentially composed of epithelium but the fibrous stroma also forms an important element in their composition. This is due to the fact that the individual epithelial cells are not held together by any intercellular material, requiring the fibrous stroma to act as the supporting framework, thus producing the alveolar arrangement so characteristic of cancer.

Seat of occurrence.—Cancer originates primarily from any tissue containing epithelium and no tissue is exempted from the secondary growth of carcinoma.

Thus it occurs as a primary growth in the skin, breast, uterus, penis, labia, œsophagus, pylorus, larynx, liver, pancreas and other epithelial tissues. The so-called primary carcinoma in non-epithelial tissues is strictly speaking endotheliomatous in nature and on account of the close histological resemblance of these two forms of new growths, they offer much difficulty in differentiating.

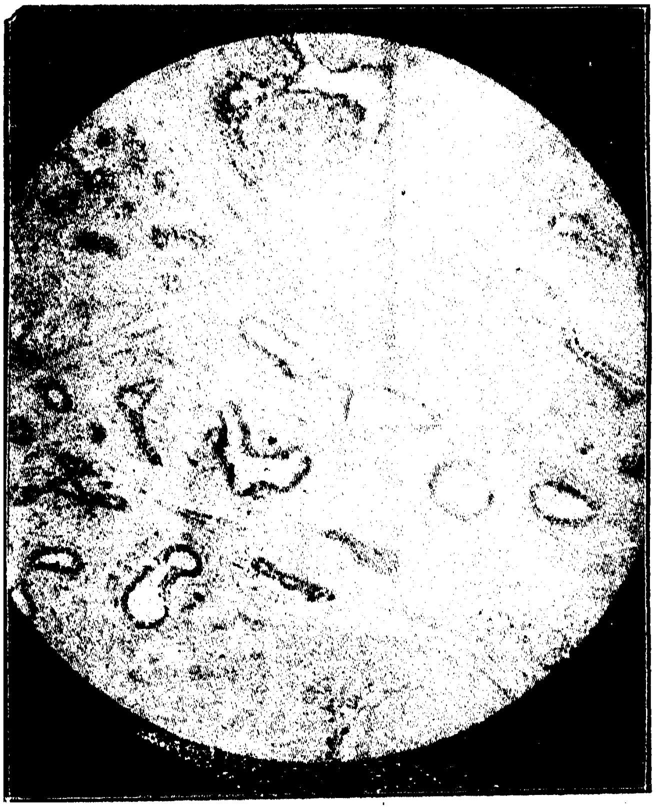

Fig. 247. Fibro-adenoma of the breast.

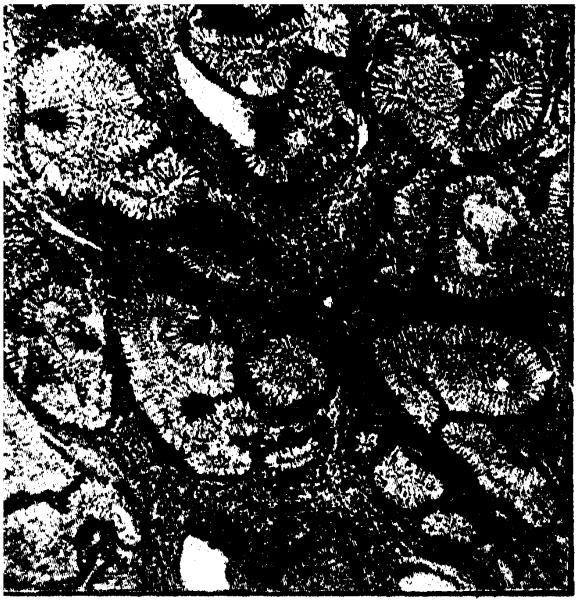

Fig. 248. Tubular Adenoma of the rectum.

Morbid anatomy.—The morphology of carcinoma is extremely variable. Carcinomata vary a good deal in size, shape and general appearance. Generally three main forms are recognised—the nodular, the ulcerative and the fungating forms. Other secondary changes, such as, necrosis, degeneration and fibrosis, cause great distortion to the appearance of the tumour. In the nodular form, they appear as irregular, more or less rounded nodules, fairly rich in fibrous tissue but not well-circumscribed, and infiltrating irregularly. When the deposition of fibrous tissue is fairly excessive, contraction takes place, by the presence of bands of cicatrices, resulting in pressure, atrophy, traction and distortion of the organ.

The ulcerative form is most common when the tumour is superficial, as in the case of skin, breast, intestine, etc.; loss of superficial tissue takes place, with the formation of shallow or deep ulcers, with indurated margins. The fungating form is characterised by the presence of masses of foul-smelling, red, easily-bleeding and suppurating tissue. The colour of these tumours is usually grey or white, but may be yellow, when it has undergone fatty degeneration, red, when hæmorrhagic and homogeneous, translucent, when undergoing colloid or mucoid degeneration. The consistency of carcinoma depends on the amount of fibrous tissue in them. Cellular tumours are soft, whereas fibrous tumours are hard and indurated.

General characters of carcinoma.—Carcinomata, when formed progressively, grow and increase in size, with rapid infiltration and disorganisation of the neighbouring structures, accompanied by certain amount of pain and loss of function. As the tumour is growing more or less rapidly, the blood vessels cannot keep pace with the growth, resulting in malnutrition and necrosis of the central portion, although the tumour is infiltrating peripherally. The central necrosis enlarges in size, until it becomes superficial, with subsequent involvement of the skin, forming an ulcerated area, through which the central necrosed tissue is evacuated. Putrefaction often takes place in the necrosed tissue, giving it a very fœtid odour. The ulcerated surface enlarges, the margin becomes indurated and hard, due to the deposition of fibrous tissue or may become soft and fungating, with occasional hæmorrhage. Sooner or later, extension takes place to the nearest lymphatic glands. In advanced stages, specially in cases of the carcinoma of the internal organs, the general health of the patient is greatly disturbed, resulting in cachexia and death.

Growth of carcinoma.—It is generally believed that carcinoma, whatever may be the size, originates from one single cell, or a group of cells which form the nucleus for the whole tumour and that all the cancer cells are derived from one or more cells and not by the fusion of several primary foci, occurring in a comparatively large area.

The cancer cells are generally atypical from the mother cell or cells and hence grow rapidly and progressively. The rate of growth varies a good deal. The expansion in size is due to peripheral extension and infiltration, with no displacement or compression of the surrounding tissues. The invasion of the tumour cell in the adjacent tissues, leaves no room for encapsulation and so these tumours are not well circumscribed. In case of loose or yielding structures, such as the areolar tissue or the lung, a capsule may appear, with minimum destruction of the surrounding tissues.

Dissemination of carcinoma.—Dissemination is the rule in carcinoma. Sooner or later the carcinoma will spread peripherally at the primary seat of origin and at the same time invade and disorganise other tissues which may be far remote from the primary growth. This dissemination in the distant parts as secondary growths is known as metastasis. The secondary growth generally represents the feature of the primary one but not in all cases. As for example, a scirrhous carcinoma of the breast may form an encephaloid one in the axillary glands or elsewhere. This usually depends on the looseness of structure of both the primary as well as the secondary growths. The secondary growth also varies a good deal from the primary one as regards the number, size, rapidity of growth, position and clinical features.

The number of secondary growths may be single or multiple often amounting to several hundreds. In case of affections of the lymph gland, the infiltration proceeds from one gland to another. In case of the liver, there may be numerous, small or large, metastatic tumours, originating secondarily from the alimentary tract.

The size of the secondary growth varies greatly. In some specimens it may be very small, often microscopical, in other instances it may be of huge size. The size of the secondary growth is usually smaller than the primary one, but that is not always the case. Rapidity of growth of the secondary tumour is also variable. The position of the secondary tumour depends on the anatomical position of the primary focus. Thus liver is the organ most commonly affected in case of primary carcinoma of the alimentary tract. The thyroid and the prostate usually give secondary metastasis in the bones. Spleen is rarely involved even in case of extreme metastasis in other organs.

Clinically, it is often found that the main manifestations are due to the metastatic growths, the primary focus being very insignificant rendering it difficult to detect, except post mortem. The opposite condition, *e.g.*, insignificant secondary growths, is also not uncommon. Dissemination may take place in either of the three following ways:—(1) Embolism, (2) Permeation of Handley, (3) Inoculation.

Embolism.—This is the commonest mode of dissemination. The metastasis of carcinoma usually takes place through the lymphatics. The embolus may consist of only one cell or a group of cells and is carried generally through the lymphatic vessels to the lymphatic glands or other structures, until it is arrested and develop into secondary growths. The emboli are necessarily microscopical and are often found lying in the capillaries of various organs simultaneously. Metastasis may also occur through the blood, specially in the alimentary tract, through the portal circulation, to the liver. Hæmatogenous metastasis may also take place by way of the lymphatics through the thoracic duct. Veins, being thin walled, may also act as the transmitting agent.

Permeation of Handley.—In this process, the cancer cells extend peripherally into the large lymphatic vessels in masses or columns, resulting in stretching and often bursting of the vessels. The interesting feature is, that the extension along the lymphatics often dies out in one direction and progresses in another. This is explained by the fact that the stretching of the lymphatic vessels by the cell masses produces certain amount of inflammation resulting in round cell infiltration and accumulation of fibroblasts. The pressure exerted by the fibroblasts, may often be sufficient to destroy the cancer cells. The cells at the margin, which escape pressure, continue to grow, with the formation of secondary growths, whenever suitable soil is reached. This is most commonly observed in the carcinoma of the breast and, although embolic dissemination may take place, Handley thinks that permeation is the main feature in such cases. Permeation always takes place in large lymphatic vessels and not in lymph spaces, thus affording greater opportunity for rapid dissemination to take place, and are best observed in the tissue, some distance away from the edge of the primary growth.

Inoculation.—This mode of dissemination is observed in cases, where the cancer cells get access to some abraded surfaces, either by contact or apposition. This is illustrated by the formation of secondary growths in the vaginal wall from primary carcinoma of the cervix uteri. Bursting of carcinomatous growths in the pleura or peritoneum leads to the dissemination of small masses of cancer cells throughout the serous cavity, either directly or by the movements of the contained organs. The invasion of the cancer cells in such circumstances will be seen to extend into the organs from without inwards, that is from the serous coat inwards towards the lumen.

This fact is of great importance to the surgeon during the surgical removal of a cancerous tissue. If some of the cancer cells are allowed to escape in the wound, either by rough handling or imperfect removal of the mass, a recurrence will invariably take place. The scalpel, needle or other instruments, contaminated with the cancer cells, may be the source of dissemination.

Structure of carcinoma.—The epithelial cells are not provided with any intercellular material, thus requiring some amount of fibrous tissue for the support of the cells themselves, as well as the nutrient vessels for the nourishment of the epithelial cells. Thus the connective tissue plays a very important part in the existence of the tumour cells. The equilibrium is often disturbed by the growth of cancer cells inside the vessels, cutting off their own nutrition, or in other instances the connective tissue grows excessively, resulting in the pressure on the cells, with subsequent degeneration and destruction.

Thus there are two essential elements in the carcinoma: one, the epithelial cells, and the other the connective tissue stroma, the latter enclosing groups of loose cancer cells. That the cells are loose and are not held up by any intercellular material is demonstrated by scraping the cut surface of the tumour, when a milky fluid—the "cancer juice," will be obtained, which when examined under the microscope will be seen to consist of cancer cells mainly. A small piece, when shaken thoroughly in a test tube with a little water, will be found to consist of the fibrous stroma only, all the cells being dislodged by shaking. When the fibrous tissue is excessive, contraction takes place, with atrophy and degeneration of the cancer cells. This accounts for the retraction of the nipple in cases of carcinoma of the breast, and the umbilication of the superficial carcinomatous growths of the liver.

The fibrous stroma is distinctly visible as white bands on the cut surface of the tumour, contrasting distinctly with the pinkish cancer cells and the surrounding tissue of the affected organ. The arrangement of the cellular infiltration into the surrounding healthy tissue is most peculiar. The extension takes place in all directions, generally along the path of least resistance, but surrounded by connective tissue stroma, either growing simultaneously, or utilising the pre-existing connective tissue of the affected organ. The result of the infiltration of cancer cells is that the whole tumour consists of a continued, branched, ramifying mass of epithelial cells, resembling the invasion of roots of a plant into the soil.

Histology.—*The cancer cells* are derived from the epithelial cells of the affected organ. The cells multiply very rapidly and infiltrate deeper and deeper into the surrounding tissues and thus proceed far away from the mother cell. The cells vary a great deal morphologically. The cells nearer to the mother cells resemble it closely, but as they proceed further and further away from the mother cell, they become more and more atypical, until the remotest cells resemble those of the embryonic type. In some forms of growth such as the columnar cell type, there is no histological variation even when they have gone far away from the mother cell, and also persist in the secondary growths. The peculiar tendencies of particular cells are retained both in the primary as well as in the secondary growths. Thus keratin formation, peculiar to the epithelium of the skin, is present in all cases of epithelioma of the skin, and the origin can be easily detected by their presence in secondary growths. In case of a secondary carcinoma of the lung, it is fairly easy to recognise the primary seat, by examination of the type of cells present, whether they are derived from the skin, breast or the intestines.

The size and shape of the cells vary a great deal according to their origin and rapid multiplication. On account of their rapid multiplication they suffer from mutual pressure and hence the shape is altered. In rare instances, one may be taken into the substance of another cell forming what is known as the "cell-inclusion" (Russel's fuchsin bodies). The cells show mitotic figures, indicating rapid multiplication of the nuclei.

The Stroma.—There is apparently no intercellular material between the cells, excepting the stroma which supports the masses or groups of the cancer cells. The stroma is usually derived from the pre-existing connective tissue and is more abundant in the tissues where connective tissue is present in large numbers normally. Although the stroma is derived from the pre-existing tissue, still a large amount of it is newly formed. This is generally observed in the lymph glands, where the connective tissue is normally scanty, but in secondary carcinomatous growths, large amounts of stroma are found to grow. This shows that both the epithelium and the stroma proliferate simultaneously and form a very important part in the growth of carcinoma. The amount of stroma varies considerably. In some instances the stroma is very scanty and the cells are very numerous, the growth being called a *soft, medullary or encephaloid carcinoma.* When the amount of stroma is excessive and the cells are few in proportion, the tumour is called *hard or scirrhous carcinoma.* The stroma is the support of the cancer cells as well as the blood vessels and is richly supplied with lymphatics.

Round cell infiltration occasionally takes place, the round cells being mainly the lymphocytes, plasma cells and a few eosinophiles. Large numbers of the polymorphonuclear leucocytes appear when the tumour has ulcerated.

Infiltration and degenerations.—*Infiltration.*—The natural peculiarity of different forms of epithelium to undergo infiltrative changes is also present in carcinoma. Thus keratinisation, with formation of " epithelial pearls," takes place in squamous cell carcinoma. The production of mucoid and colloid material—the peculiarity of the columnar cells and the glands is found in the columnar cell carcinoma. Fatty changes are observed in carcinoma of the breast. Calcification is frequent in the " epithelial pearls." Serous infiltration is also common. *Degeneration.*—The disposition of the stroma and its vascularity are often responsible for malnutrition, resulting in ulceration and other degenerative changes. Infection of the ulcerated areas often leads to extensive inflammation and necrosis.

Varieties of carcinoma.—Carcinoma originates from three main sources, according to the peculiarities of the epithelium.

(1) *Squamous-celled carcinoma,* also called squamous-celled epithelioma or epidermoid carcinoma. Rodent ulcer is a form of squamous-celled carcinoma. (2) *Columnar-celled carcinoma,* also called cylindrical epithelioma, malignant adenoma, adenocarcinoma. (3) *Glandular-celled carcinoma,* also called spheroidal-celled, polyhedral or cubical-celled carcinoma. (4) *Chorionic epithelioma,* also called syncytioma malignum. This originates from fœtal tissue and hence is generally regarded as a form of teratoma.

The following terms are applied to any of the above varieties of carcinoma according to the particular characteristics.

Scirrhous or hard—when the stroma is excessive. *Encephaloid, soft or medullary*—when stroma is scanty. *Colloid or muccid*—when undergoing mucoid degeneration. *Primary*—when originating primarily. *Secondary or metastatic*—when originating secondarily from a primary focus. *Recurrent*—when recurs after excision.

SQUAMOUS CELL CARCINOMA

(*Squamous celled epithelima, Epidermoid carcinoma*).

The tumours of this group occur upon the skin and the mucous membranes lined by squamous epithelium.

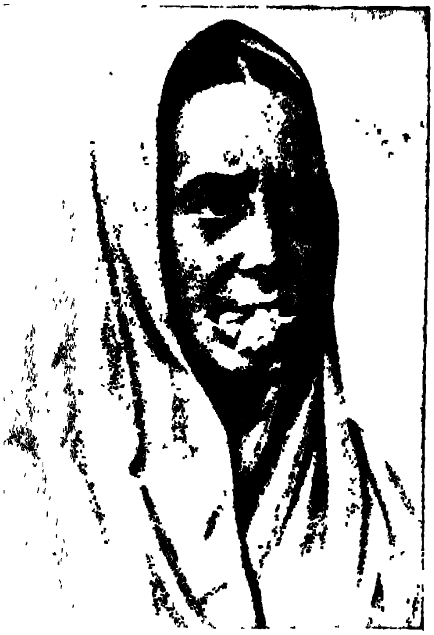

Fig. 249. Cancer of the lip (From the Pathological Museum, Cal. Med. Col.)

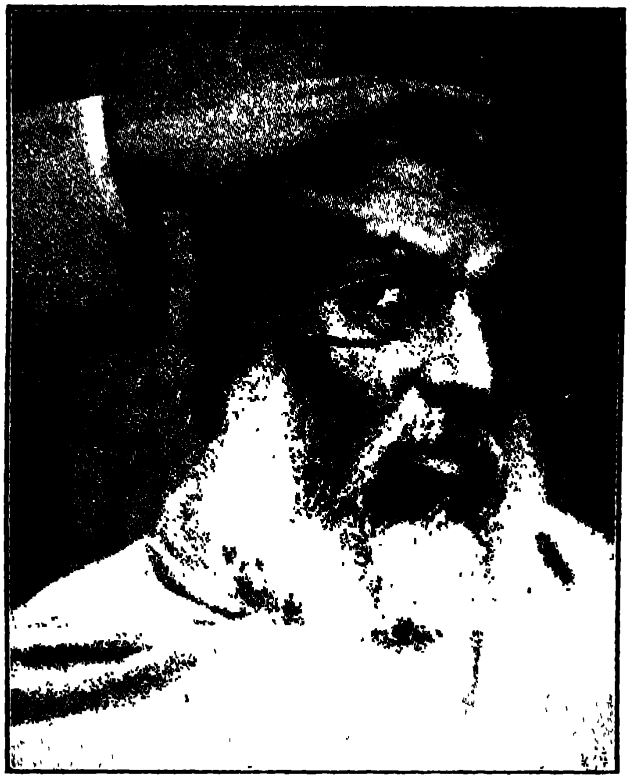

Fig. 250. Cancer of the conjunctiva. (From Dr. Charubrata Roy's collection).

Seat of occurrence.—They occur upon the skin, especially about the lips, eyelids and other parts of the face, tongue, mouth, pharynx, œsophagus, cardiac end of the stomach, larynx, labia, penis, vagina, cervix uteri, bladder, ureter, pelvis of the kidney and the anus.

Morbid anatomy.—The occurrence of carcinoma of the skin generally starts from some mechanical or other irritations and is common in those parts of the body which are more liable to such injuries. The carcinoma may also be preceded by some form of benign often insignificant growth or other conditions of the skin, *e.g.*, warts, scars, sebaceous cysts, fissures, etc. They generally occur in those parts of the body where different types of epithelium meet, *e.g.*, the margin of the lips, anus, cervix uteri, etc. The surface ulcerates early and is liable to infection resulting in inflammation and necrosis. These tumours are never encapsulated nor well-circumscribed.

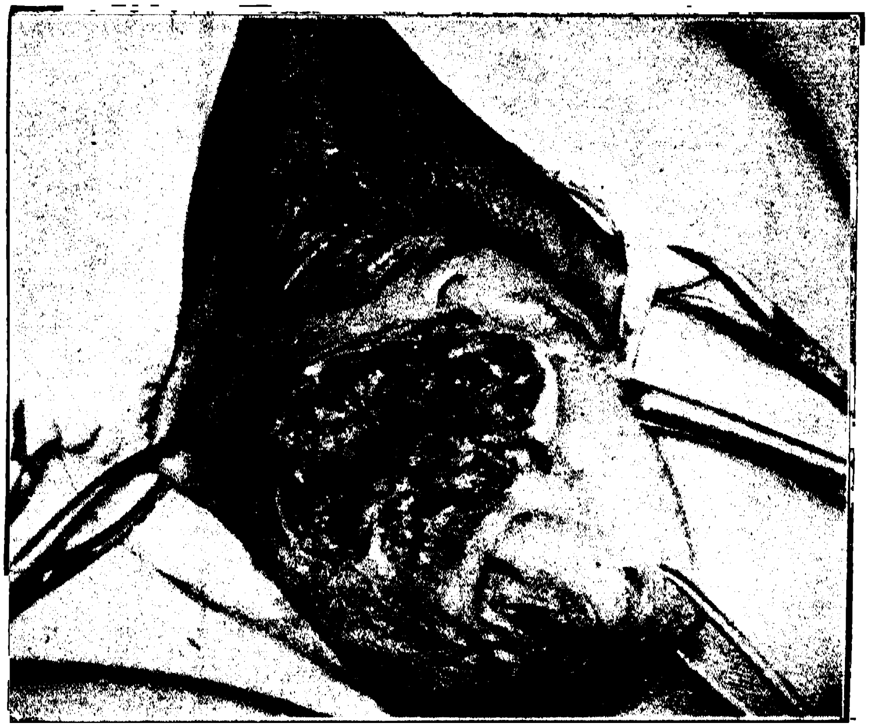

Fig. 251. Primary Epithelioma of Vagina--Prolapse of uterus for 25 years since birth of last child. (From Mr Subodh Datta's collection.)

Pathological histology.—The histological appearance is characteristic. The tumour consists of squamous epithelium arranged in masses invading the deeper layers with disorganisation of the basement membrane. The newly formed cells resemble closely the mother cells but as they grow deeper, degenerative changes are observed until the oldest cells, completely degenerated, form the "epithelial pearls" or the "cell nests." Thus the old cells, corresponding to the ordinary superficial layer of the skin—the stratum corneum, generally occupy a central position in the tumour and are larger than the peripheral cells undergoing keratosis, the characteristic of the squamous cell carcinoma. The "cell nests" consist of concentric groups of cells with keratosis in the centre.

The peripheral cells are present in large masses, stain deeply and are smaller than the old and the central cells. They are joined together by means of a large number of protoplasmic outgrowths or "prickles" on the surface, for which the term "prickle cell" is applied. The cells are loaded with black staining droplets of eleidin. This typical appearance is not present everywhere and is greatly modified when the tumour is growing rapidly. In rapidly growing forms the rounded type of cell may be flattened and distorted by their mutual pressure. The cells, may be very small, or very large, multinucleated forms with numerous cell inclusions may also occur.

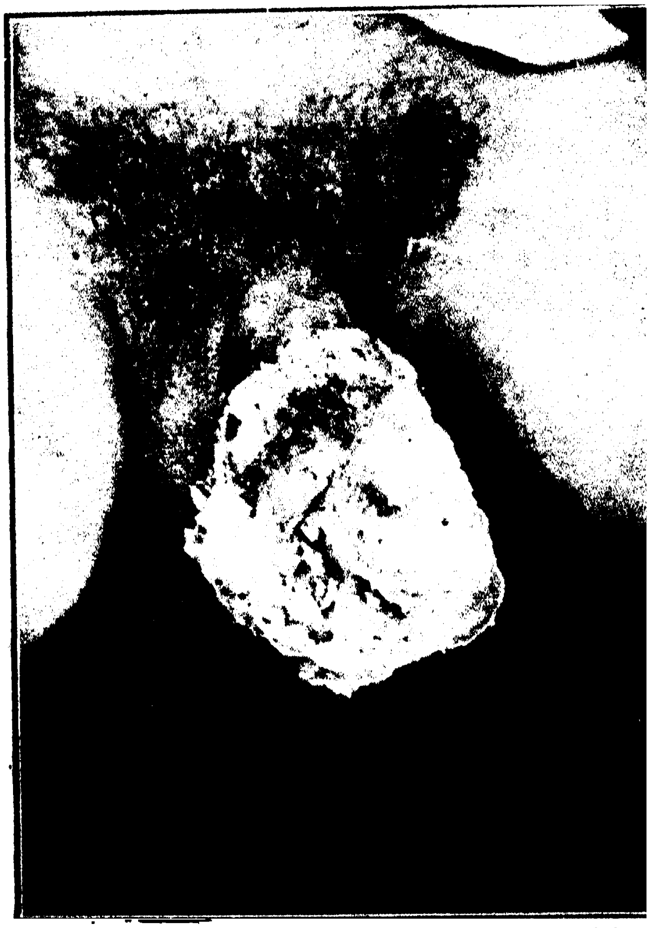

Fig. 252. Cancer penis after circumcision. (Mahomedan boy of 17)
(From Carmichael Medical College Hospital.)

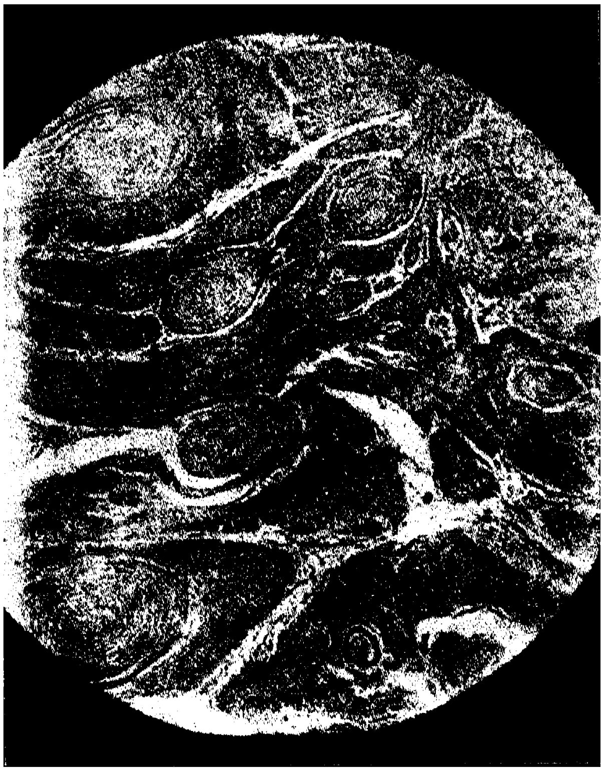

Fig. 253. Squamous-cell carcinoma showing the characteristic "Cell nests."

The keratinisation and the "prickle cells," the two main characteristics of squamous cell carcinoma, are often absent in some instances, specially in cases of carcinoma of the pharynx and œsophagus and in

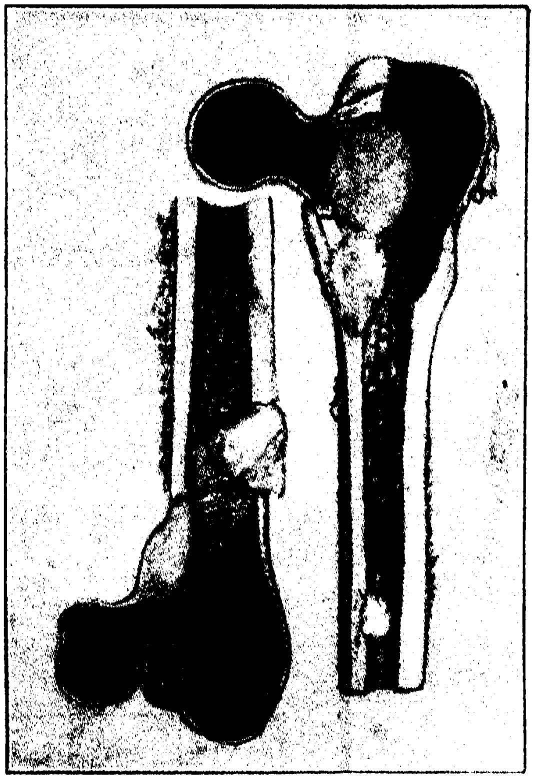

Fig. 254. Secondary Cancer of femur with spontaneous fracture. From Carmichæl Med. Col. Path. Museum.

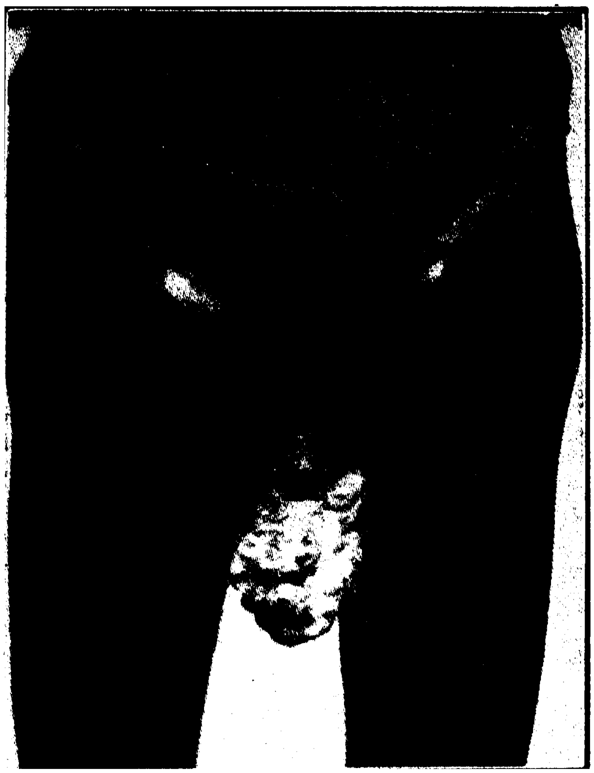

Fig. 255. Cancer penis with secondary involvement of inguinal glands. (From Dr. Nagendranath Das's collection.)

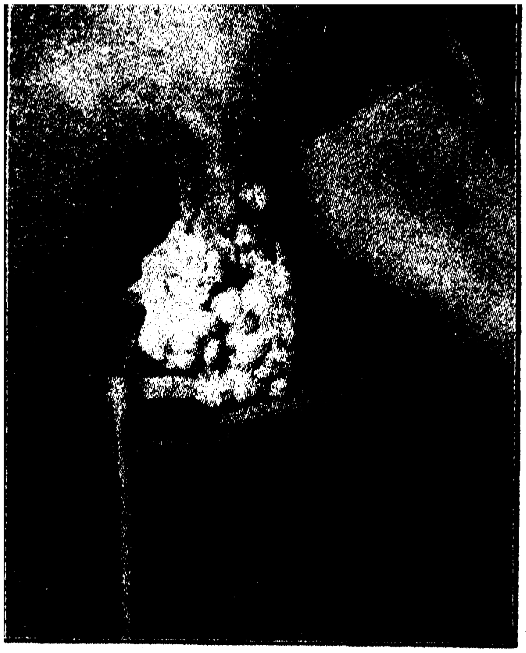

Fig. 256. Cancer penis. (From Dr. P. K. Mitra)

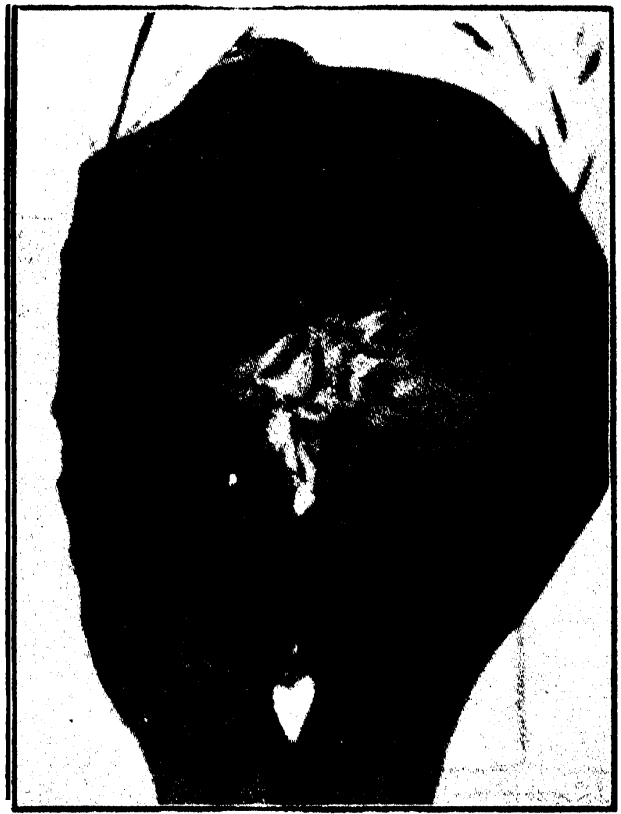

Fig. 257. Cancer of the rectum. (From Dr. Nagendranath Das's collection.)

rodent ulcer. The latter condition is most peculiar, deserving a separate consideration. The stroma is present, supporting the masses of cells and carries the blood vessels and the lymphatics. Round cell infiltration is present in considerable degree in the stroma. Presence of a very large number ot plasma cells is common.

Rodent Ulcer

Rodent ulcer is known as Jacob's ulcer, basal-celled carcinoma, carcinoma basocellulare, "noil me tangere," epithelioma exudus, cancroid, etc.

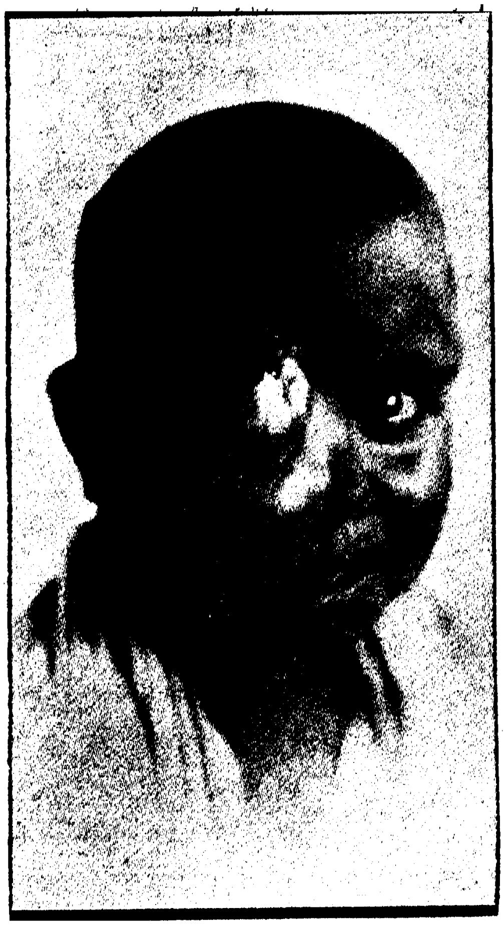

Fig. 258. Cancer of the right eyelids. (From Calcutta Medical Journal.)

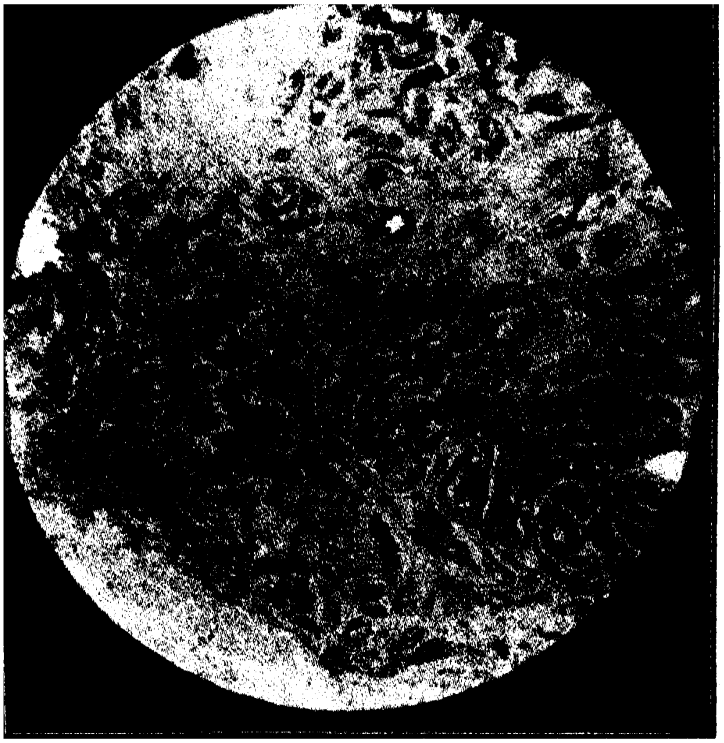

Fig. 259. Scirrhous Cancer of the Breast.

The origin of rodent ulcers is not definitely known. It is generally believed that they originate either from the lowermost or basal layer of the epithelium ot the epidermis (carcinoma basocellulare) or from the hair follicles or sweat or sebaceous glands. Some authorities regard them to be endothelial in nature.

Seat of occurrence.—They commonly occur about the forehead, eyelids, nose, ear and other parts of the face. They occur rarely in other parts of the body such as the back.

Morbid anatomy.—They start in the form of a small papule which grows slowly but progressively. Ulceration takes place early with much loss of tissue. They are relatively benign, and the tendency towards metastasis is rare. It has been observed, that any rough handling with these growths, often changes their benign character and they commence to grow very actively, with much deformity and metastasis. For this reason, the older surgeons termed it "*noli me tangere*" (touch me not).

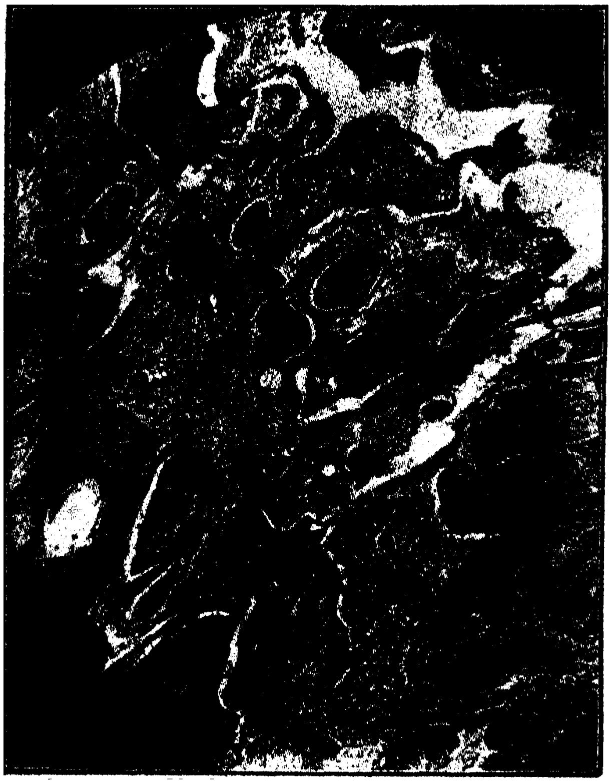

Fig. 260. Rodent ulcer (Low power).

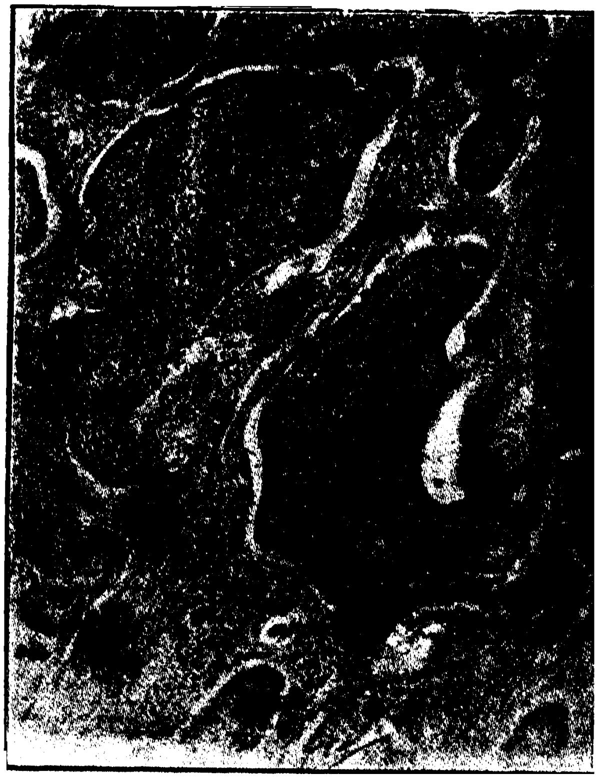

Fig. 261. Rodent ulcer (High power).

Pathological histology.—The histological appearance is characteristic. Unlike the other forms of squamous-cell-carcinoma, the downward growing strands are seen to reach to about the same level, in the form of irregular papilla-like collections of squamous epithelium, very often cylindrical in type. The line of demarcation between the epithelium and the stroma is more or less sharply defined, indicating a benign type of growth. The epithelial cells are closely packed, smaller than those of epithelioma, and contain relatively scanty protoplasm. The prickle cells are absent and, as they are growing from the basal cells, they show no tendency to keratinisation and formation of "epithelial pearls."

Adamantine Epithelioma or Adamantinoma

These grow in the substance of the jaw bones, at the base of the tooth, and are derived from the epithelial structure concerned in the formation of the enamel. They originate as hard masses, displace the tooth and disorganise the bone.

They are benign in nature, but resemble epithelioma histologically. Numerous cavities appear in the epithelial masses. The stroma is abundant and often contains bone.

COLUMNAR-CELL CARCINOMA

(*Cylindrical cell carcinoma, duct carcinoma, malignant adenoma or adenocarcinoma.*)

These originate from all mucous membranes in which the epithelium is cylindrical in form, and also from the ducts and glands lined by similar epithelium. They are commonly found to grow in the

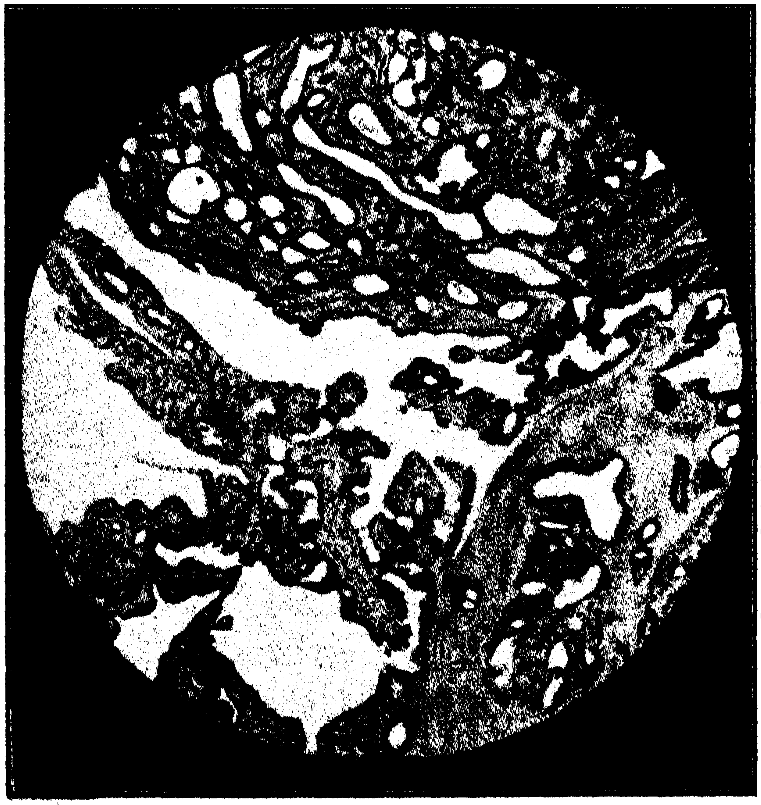

Fig. 262. Malignant adenoma

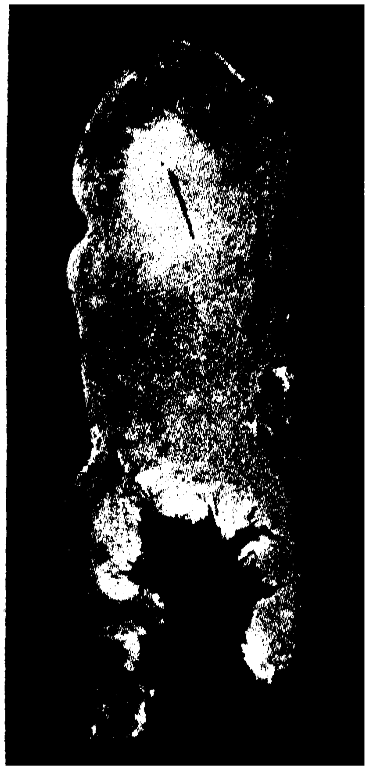

Fig. 263. Total frozen section of the uterus showing carcinoma of the cervix and vagina.

stomach, gall bladder, bile-ducts, pancreas, large intestine, body of the uterus, etc. Microscopically they resemble glandular tissue, with definite alveolar arrangement, although irregularly. The glandular arrangement is often modified in the metastatic growths, but the presence of the columnar cells can be made out on careful examination in such secondary growths.

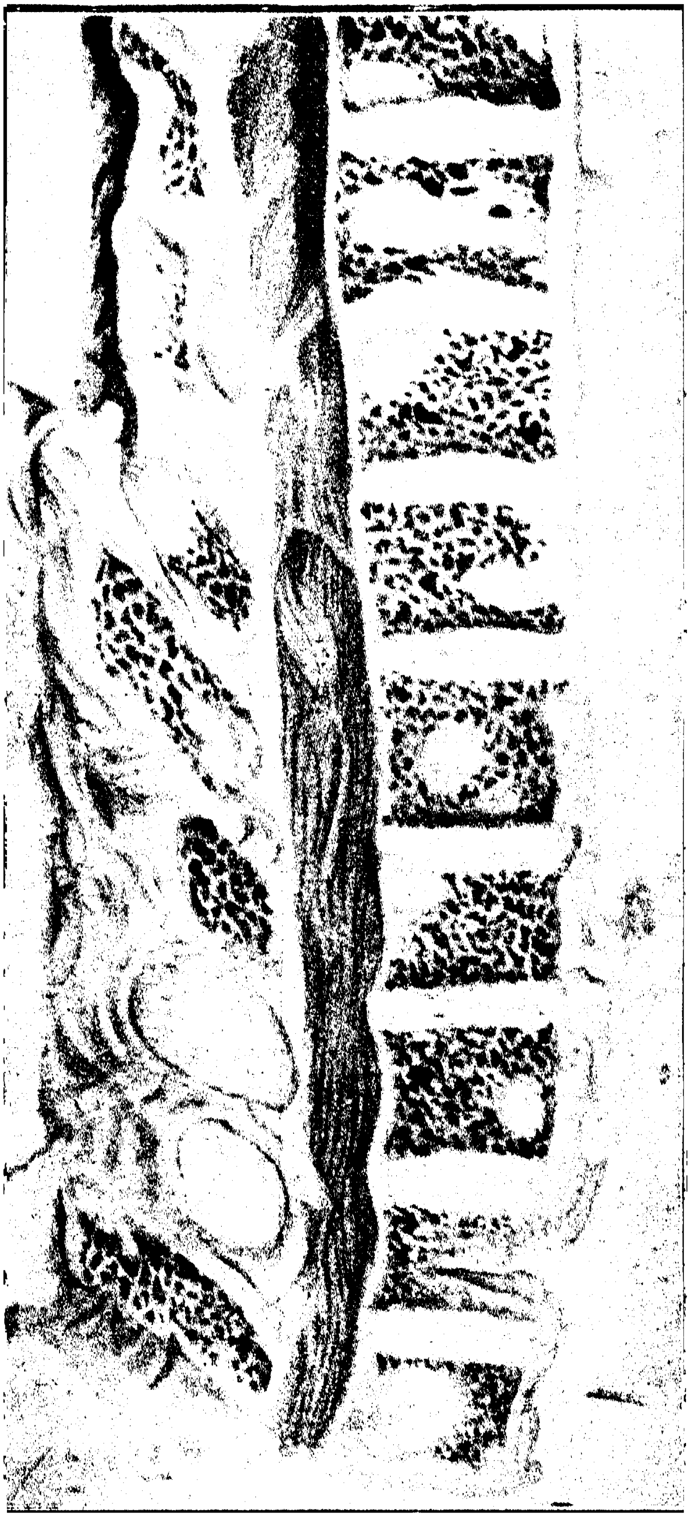

Fig. 264. Cancer of the vertebra.
(From Pathological Museum, Cal. Med. College.)

GLANDULAR-CELLED CARCINOMA

(*Spheroidal-celled Carcinoma.*)

This form of tumour is most commonly found in the breast. The cells are generally spheroidal in shape but often become polygonal due to mutual pressure. The cell possesses an abundant cytoplasm with a deeply stained nucleus. The cells are grouped in columns or in solid alveoli, according to the organ affected. Thus the epithelial cells are arranged—(*a*) In the form of ducts or tubules, *e.g.*, in breast, mucous and salivary glands, etc. (*b*) In the form of solid masses, *e.g.*, in the adrenal cortex, (*c*) In the form of lining of spaces, *e.g.*, in the thyroid, or (*d*) In the form of a combination of the above three types.

The stroma is variable, when it is abundant and dense, the growth is called scirrhous and when scanty and delicate, medullary or soft.

CHORION-EPITHELIOMA

(*Syncytioma malignum.*)

This originates from the epithelium of the fœtus and so must be in connection with pregnancy. It has been reported recently, that chorion-epithelioma can originate from organs other than the female generative organs. Several cases have been reported by Pick occurring in the adult males. It may occur during or after a normal pregnancy, but usually occurs after an abortion or is preceded by a hydatidiform mole. During pregnancy, there is some amount of invasive growth of the chorionic villi into the uterine wall, but this ends harmlessly with the disappearance of the cell of the chorionic villi from the tissue. Occasionally this invasive process progresses

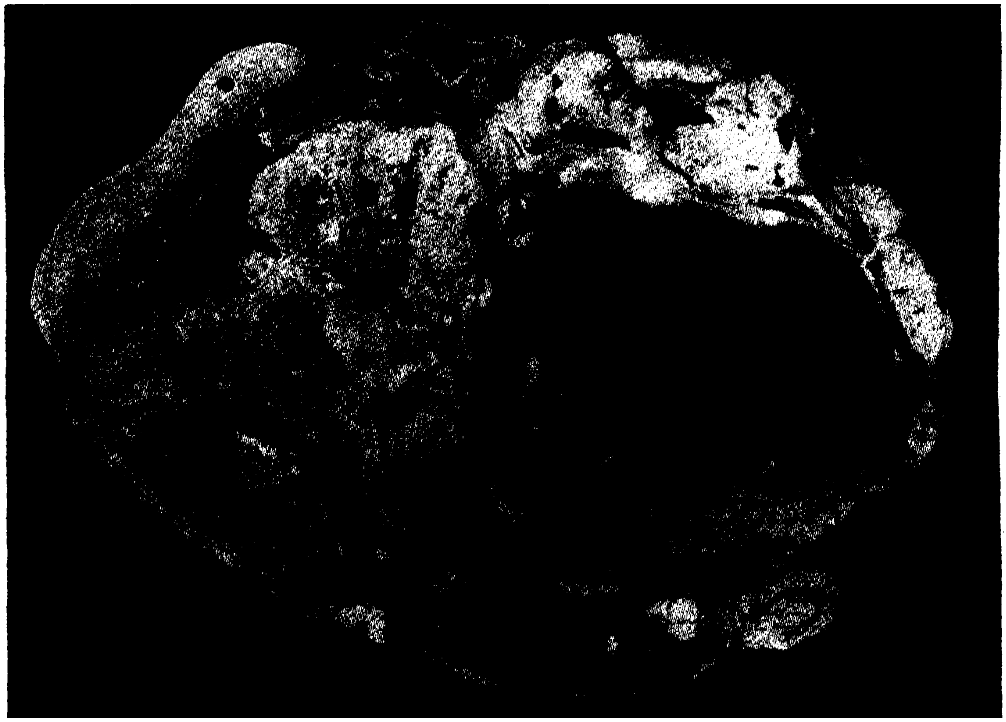

Fig. 265. Total frozen section of carcinoma of the kidney.

and forms what is known as the hydatidiform mole. In rare instances the invasion is excessive and gives rise to a tumourlike mass with extensive metastasis, hæmorrhage and malignancy. This condition is known as the chorion-epithelioma and its occurrence is proportional to the number of previous pregnancies.

The cells which cover the chorionic villi are generally of two types—(*a*) Langhan's cell layer, and the (*b*) Syncytial layer. Both of these cells are derived from the fœtal ectoderm and thus are the derivatives of another individual which led some workers to classify this form of tumour under teratomata.

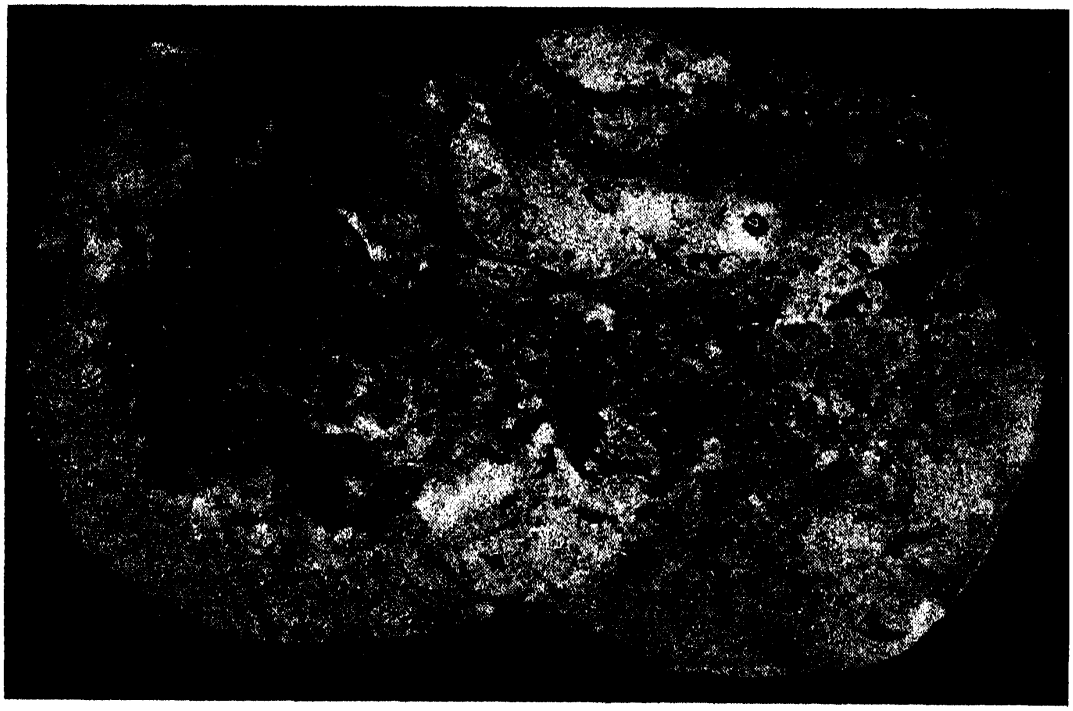

Fig. 266. Total frozen section of adenocarcinoma of the Kidney. Portion of healthy kidney is seen on the left.

Morbid anatomy.—The growth is fleshy and extremely hæmorrhagic. Metastasis takes place early and with great rapidity, owing to the early invasion of the blood channels. Secondary deposits occur commonly in the lungs and the liver. It is extremely malignant but spontaneous disappearance and recovery is not uncommon.

Histology.—The two main types of cells are quite distinct. The Langhan's cells are smaller, sharply-outlined, more regular, cuboidal or polygonal in shape, with pale and almost clear cytoplasm and a large nucleus. The syncytial cells, on the other hand, consist of large masses of solid areas of deeply stained protoplasm containing numerous large oval nuclei. The protoplasm is irregular in outline and often contains vacuoles. There is no definite stroma and the blood vessels are deficient partly by imperfect development and partly by the invasion of the tumour cells inside the vascular channels which are distended and thus afford ample opportunities for rapid and early metastasis, extensive hæmorrhages and degenerative changes due to malnutrition. Formerly, it was believed to have originated from the uterus, and was termed deciduoma malignum, but, now it has been confirmed that the tumour originates from the fœtal ectoderm of the placenta and not trom the material tissue.

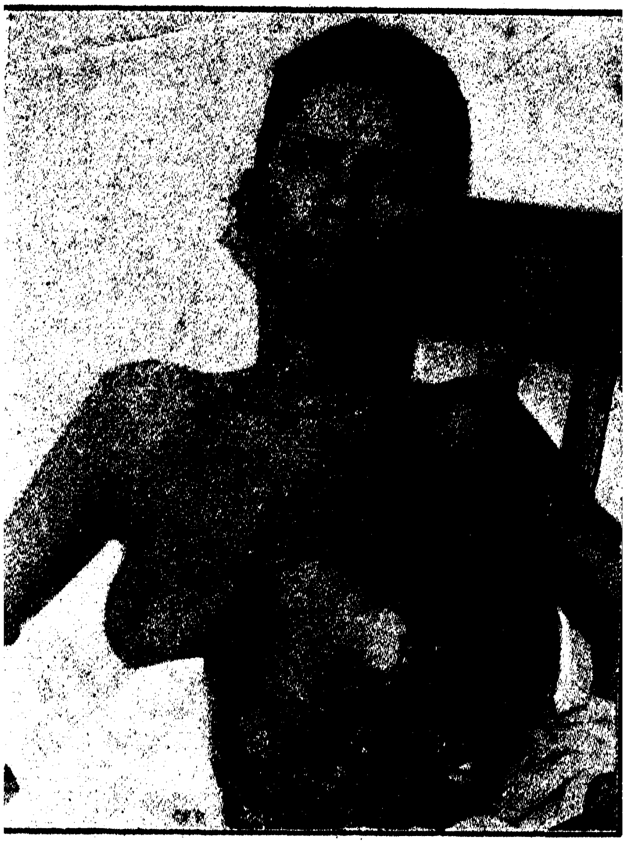

Fig. 267. Cancer of the breast (From Dr. P. K. Mitra).

Krukenberg's tumour.—(*Fibro-sarcoma ovarii mucocellulare carcinomatodes*).—Krukenberg observed, in certain cases of cancer of the ovary, sarcomatous change of the stroma, and the cancer cells showing mucoid changes. The above synonym describes the histological character. This tumour generally affects both the ovaries and is generally associated with (or secondary to) cancer of the stomach.

EXPERIMENTAL STUDY OF CARCINOMA.

The study of carcinoma is considered to be of great importance both from clinical as well as from pathological points of view. The clinician very often comes across with carcinoma, but its wide distribution and rapidly changing character afford difficulties for its proper study. When a case comes under his observation, the clinician has got to depend mainly on the observation of the patient, as regards the previous behaviour of the tumour and also remains in the dark about its future occurrences. Only by means of continued and minute observation of a series of similar growths, the clinician will be able to make a continuous chain as regards the life history of a particular form of carcinoma. Still, some links of the chain remain to be filled up by mere speculation. Under these circumstances the experimental production of carcinoma, if successful, in a laboratory animal, will afford immense opportunity for both the pathological as well as the clinical study of the affection. For a long time, the experimental transfer of carcinoma from one individual to another was attempted but with partial success, and only in recent years the systematic study on lower animals has attracted the serious attention of the pathologists and the work is already far advanced. It has been found that a large number of different animals—mammals, birds, even amphibians and fish is liable to the same morbid growths as the human beings. Of the laboratory animals, mouse is very commonly selected on account of cheapness, small size and ease to handle.

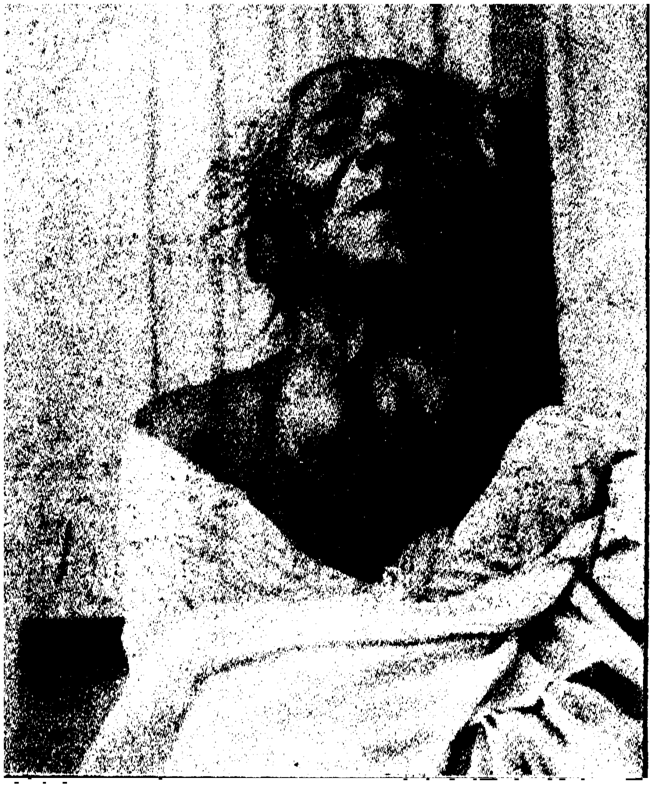

Fig. 268. Malignant adenoma thyroid (From Dr. P. K. Mitra)

Method of transplantation.—This is done by the two following methods:—(1) The graft, as an intact fragment of tissue, is introduced into a small pocket in the subcutaneous tissue made by a pair of scissors. (2) The graft is emulsified in saline and introduced into the muscles, peritoneal cavity or into the viscera. The advantage of the second method is, that the deeper organs can be approached and the dose can be accurately regulated.

The success of the transplantation depends on the following factors. (*a*) Vitality of the cells introduced—The cancer cells are very susceptible to rough handling and also to drying, heat and exposure to certain chemicals, X-rays and radium. In order to be successful, the cells must be living. (*b*) The dose of the inoculated material—It has been observed that a small dose is more successful than a large one (*c*) The susceptibility of the inoculated animal. Although animals suffer, either spontaneously or experimentally, from cancerous growth, still the cells of one animal have got the power to destroy cells of an animal of different species (cytolysins). This renders the successful inoculation of the mouse from carcinomatous tissue of human beings, difficult. But once a mouse is successfully inoculated, cancer can be transmitted from generation to generation, provided the animals belong to the same species. (*d*) Asepsis is absolutely necessary for the success.

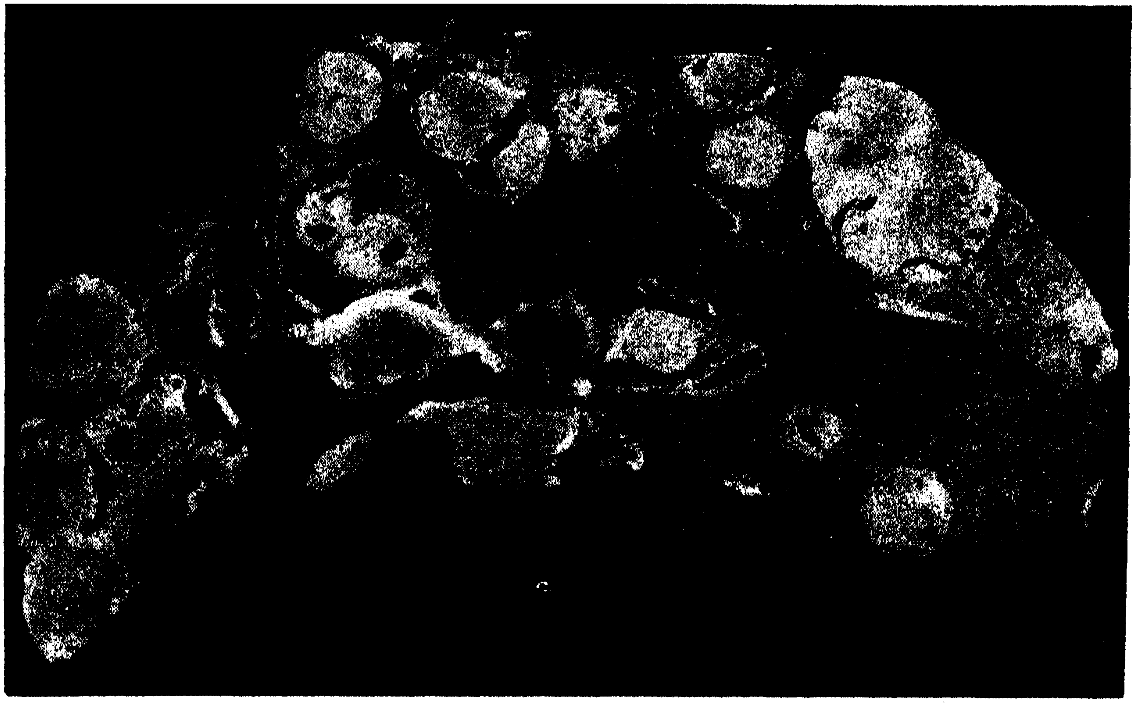

Fig. 269. Total frozen section of the lung showing multiple nodules of metastatic chorion-epithelioma.

CHANGES OBSERVED.—The graft, when introduced into the tissue, owing to irritation, leads to the accumulation of leucocytes in and surrounding it and within a period of about 24 hours it is surrounded by fibroblasts. The stroma of the graft shows evidence of degeneration which progresses and reaches its maximum in about three days. During this period, the newly formed fibroblasts surrounding the graft attempt to enter into it, and in the following days completely replace the degenerative stroma of the graft. Thus in about two weeks the graft acquires a new well vascular stroma from the connective tissue of the host. The cells of the graft survive the degenerative changes of its original stroma and now continue to grow and behave exactly as the spontaneous growth. Sometimes it is observed, that although the graft is successfully inoculated, yet it is absorbed subsequently, leaving the animal more resistant to a further inoculation. It has also been found, that if an animal is inoculated with normal tissues, such as the emulsion of normal organs, or of the embryo of the same animal or defibrinated blood, it is rendered resistant against any inoculation with tumour graft. On the other hand, the tumour graft can be transplanted in a susceptible animal, from one to another, for hundreds of generations, although the first inoculated animal and its offsprings have long died of old age. This illustrates how a tumour cell can remain alive and thrive for generations, the so-called artificial immortality.

CHAPTER XXX.

THE TERATOMATA

The teratomata originate from all the three blastodermic layers. They occur commonly in the forms of the dermoid cysts and the "mixed parotid tumours." These tumours often become cystic and contain the following elements. *Epiblastic elements.*—The skin, hair follicles, sweat-glands, sebaceous glands, hair, teeth, nerve fibres, mucous membrane, mucous glands, cholesterin, etc. *Mesoblastic elements.*—Areolar tissue, muscles, cartilage, bone, etc. *Hypoblastic elements.*—Endothelium, etc.

CYSTS.

Cysts are rounded sacs with a fibrous wall lined by endothelium or epithelium and containing a fluid or semisolid material, the character of which varies according to the tissue affected.

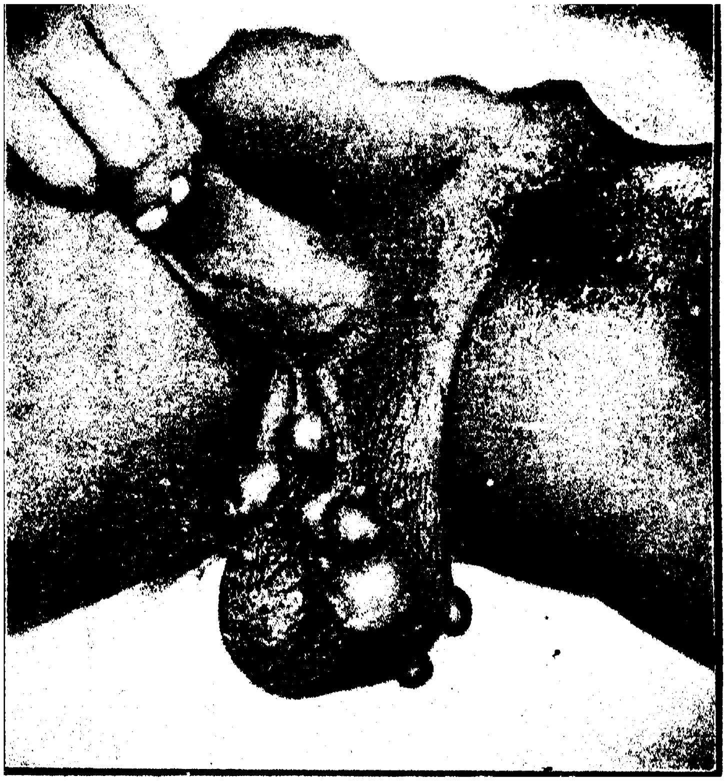

Fig. 270. Sebaceous Cysts of Scrotum.
(From Carmichæl Medical College Hospital).

Classification.—1. Cysts originating from distension of pre-existing spaces.—

(*a*) *Retention cysts*—These are caused by the retention of the normal secretory products, due to the obstruction of the ducts. They are common in case of secretory epithelial glands and so the wall of the cyst is lined by epithelium. Examples.—Sebaceous cyst, mucous cyst, lachrymal cyst, pancreatic cyst, ranula (cyst from the salivary gland), etc.

(*b*) *Exudation cysts*—These occur in cavities with no ducts. They may be of inflammatory origin also. The walls may be lined with epithelium or endothelium according to the structure involved Examples.—Ovarian cyst, thyroid cyst, etc.

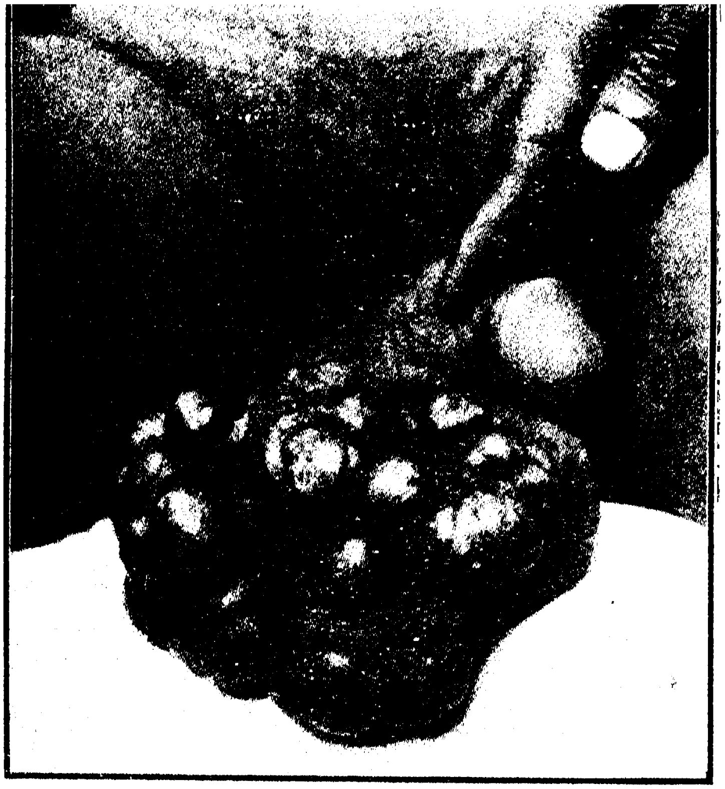

Fig. 271. Sebaceous cyst of Scrotum (From Dr. D. C. Chakrabortty)

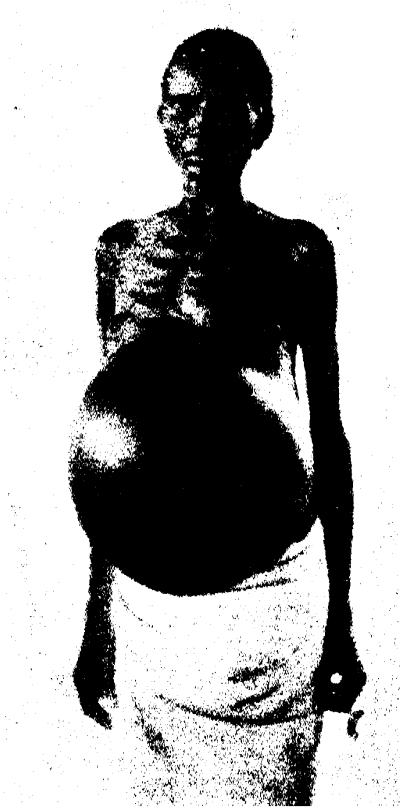

Fig. 272. Ovarian Cyst. (From Dr. Kedarnath Das's case).

II. Congenital cysts.—

(*a*) *Dermoid cysts.* —These occur generally in connection with the ovaries and are characterised by the presence of a wall lined with skin and containing such structures as hairs, nails, sebaceous material, desquamated epithelium, cholesterin crystals and fat droplets. They may be unilocular or multilocular.

(*b*) *Cysts originating from the pre-existent fœtal structures, e.g.*, thyrolingual cyst, branchial cyst, parovarian cyst, fibro-cystic disease of the testes, encysted hydrocele, etc.

III. Cysts of new formation.—

(*a*) *Serous cysts.*—These are generally produced by the accumulation of the inflamed products in the tissues

(*b*) *Degeneration cysts or spurious cysts.*—These occur in the tissues, due to the degenerative or necrotic process, resulting in liquefaction of the tissues. These are commonly found in old abscesses and neoplasms.

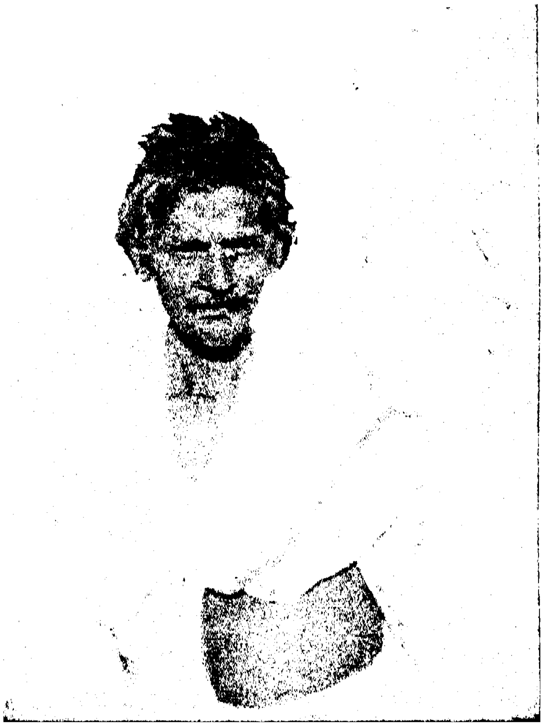

Fig. 273. Lachrymal cyst.
(From Dr. Baidyanath Bhaduri's case).

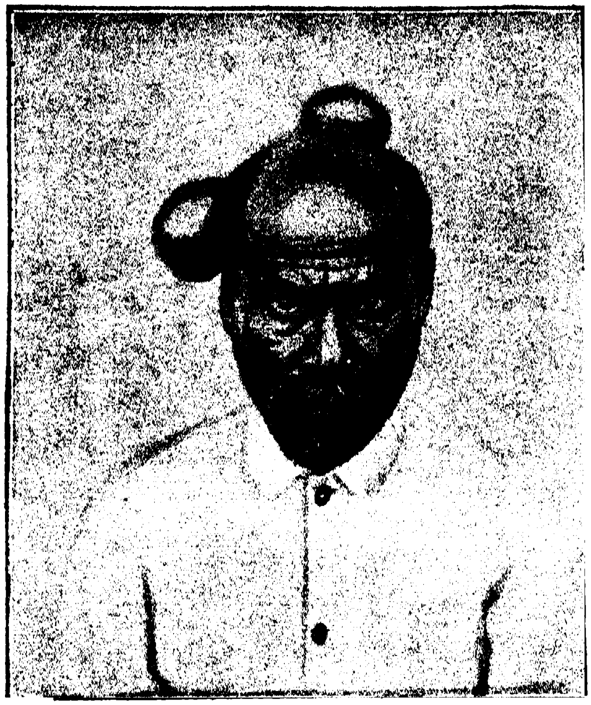

Fig. 274. Sebaceous Cysts.
(From Carmichæl Medical College Hospital).

(*c*) *Blood cysts.*—These are due to the extravasation of blood, either in closed cavities or in the tissues, *e.g.*, hæmatocele, etc.

(*d*) *Cystic tumours or cystic adenomas.*—Originating from the proliferation of mucous membrane, glands, etc. *e.g.*, in cystic tumour of the ovary, thyroid, kidney, etc.

IV. *Parasitic cysts.*—These are produced by the irritation of some parasites, the most common instance being the hydatid cyst caused by the Tænia echinococcus.

CHAPTER XXXI

DISTURBANCES OF CIRCULATION

Disturbances of circulation lead to the disturbance of the mechanism which (*a*) supplies the parts of our body with the nutritious material, oxygen, salts, etc., (*b*) removes all the excretory products of the body, and (*c*) distributes the internal secretions, ferments and similar bodies to the respective organs. The disturbances of circulation may be general or local, according to the part affected.

General Disturbances of Circulation

This takes place as a result of (1) Disturbance of the activity of the heart, (2) Disease of the vascular system; (3) Changes in the quantity and quality of the blood.

I. Disturbances of Circulation as a Result of Disturbance of the Activity of the Heart.

The disturbances are mainly due to the conditions which lead to the disproportion between the force of the heart and the peripheral resistance. In cases of increased resistance, *e.g.*, in valvular diseases, the heart (either the isolated chambers or all the chambers as a whole) becomes dilated or hypertrophied and both followed by circulatory disturbances. The capacity of the heart to dilate or hypertrophy depends on the condition of the heart muscle. The disturbances of the muscular action of the heart alone may also lead to gross general circulatory disturbances. These are (*a*) Acceleration of the heart (*tachycardia*); (*b*) Slowing of the heart (*bradycardia*); (*d*) Irregularity of the heart (*arrythmia*).

So long as the rate of contraction is not considerably accelerated or slowed, there is no appreciable disturbance of the circulation, but, when they are enormously high (*e.g.*, in paroxysmal tachycardia, the rate rising to 300 per minute) or exceedingly slow (*e.g.*, in Stokes-Adams syndrome, the rate being 20-40 per minute), there is gross disturbance in the form of general stasis of blood. This takes place mainly in the dependent parts of the body (*hypostasis*), the upper parts becoming anæmic.

Some Post Mortem Findings in the Heart of Cases from Heart Failure.

1. In cases dead from heart failure the presence of the relaxation of the heart muscle is not the sufficient indication for the weakness of the heart during life, as it is often a post mortem manifestation.

A fatty heart was formerly always identified with weakness of the heart, but we now know that the most advanced fatty changes of the muscles can be associated with normal function. Lubarsch and Aschoff believe that the fatty changes of the heart muscles are the result and not the cause of the weakness of the heart. In many cases of heart failure, the intracardiac nervous system, just as the conducting bundles, play a very important part.

2. In many cases, definite anatomical changes, *e.g.*, severe arteriosclerosis of the coronary arteries, are present.

II. Disturbances of Circulation as a Result of Diseases of the Vascular System.

The vascular system, especially the function of the musculature of the vessels, depends mainly on the influence of the nervous regulations (vasoconstrictors and vasodilators), regulated by the vasomotor centres in the medulla. Any irritation of the vasomotor centre, either by toxic or psychical causes, leads to *increased vascular tone* throughout the body, which leads to increased work of the heart and, if persistent, hypertrophy of the muscles.

Loss of Vascular Tone, due to the paralysis of the vasomotor centre, *e.g.*, in diphtheria, pneumonia or other toxins, leads to fall of blood pressure and collapse (the so-called hæmorrhage inside the blood-vessels). The phenomena of shock may be included in this group.

Diminution of Elasticity leads to the rigidity of the vascular wall and ultimately dilatation of the vessels. This leads to the compensatory growth of the intima, *i.e.*, arteriosclerosis (Thoma). Arteriosclerosis of the aorta or other bigger arteries causes very little disturbance, in comparison to the middle-sized or smaller arteries, which offer increased resistance to the circulation, followed by high blood pressure, hypertrophy and dilatation of the heart. Similar results also take place in case of rigidity of the small vessels.

III. Disturbances of Circulation as a Result of the Change in the Quantity and Quality of Blood.

This may be due to—

(*a*) *Quantitative changes.*—(*i*) Increase in the total quantity of blood, *e.g.*, in so-called plethora vera (polycythemia rubra); result—increased B.P., hypertrophy of heart. (*ii*) Increase in the quantity of serum, *e.g.*, in so-called plethora serosa (in cases of chronic nephritis); result—transudation, œdema. (*iii*) Decrease in the total quantity of blood, *e.g.*, in anæmia due to hæmorrhage (injury or operative) either external or internal. Result—fall of blood pressure, anæmia of brain. (*iv*) Relative increase in the amount of fluid without increase of the volume of the blood, the so-called hydræmia, *e.g.*, after saline infusion. (*v*) Decrease in the quantity of serum, *e.g.*, in so-called anhydræmia (concentration of blood) in cases of cholera, severe diarrhœa, etc. Result—slowing of circulation, cyanosis, cold extremities. etc.

(*b*) *Qualitative changes, e.g.*, in chlorosis, pernicious anæmia, leukæmia, etc.

We know very little as to how these influence the general circulation.

Local Disturbances of Circulation

Local disturbances of circulation depend on— (*i*) Arterial hyperæmia; (*ii*) Venous hyperæmia; (*iii*) Local anæmia or ischæmia.

Hyperæmia or congestion is a circumscribed accumulation of blood inside the blood-vessels. The term congestion is applied to the condition due to dilatation of the capillaries mainly.

ACTIVE HYPERÆMIA

Etiology.—This usually depends on vasomotor influences upon the arteries:

(*a*) *Direct*—due to paralysis of the musculature (myoparalytic or atonic hyperæmia), *e.g.*, by thermal (erythema solaræ), mechanical (rubbing, etc.).

(*b*) *Indirect*—due to nervous influences on the musculature leading to its paralysis (neurotic hyperæmia). This may be (*i*) *Neuroparalytic*— This is experimentally proved by Claude Bernard who found hyperæmia and redness of the ear or tongue of rabbits after section of the cervical sympathetic and N. lingualis respectively. In human beings, too, hyperæmia and redness appear on the face in cases of injury of the cervical sympathetic, *e.g.*, pressure by tumour or inflammation of the neighbouring structures. (*ii*) *Neurotonic or irritative hyperæmia.*—Experimentally, Claude Bernard found, by irritating the chorda tympani, hyperæmia of the submaxillary glands and excessive salivary secretion. Irritation of the N. errigentes produces erection of penis. In human beings, this is exemplified by the conditions, *e.g.*, herpes zoster, erythromelalgia, etc. (*iii*) *Reflexive.*—*e.g.*, in anger, blush of shame, roseola infantilis (during dentition).

Morbid Anatomy.—

I. *Evidence of active hyperæmia during life.*—(1) The part is intensely red which disappears on pressure, only to reappear as soon as the pressure is released. (2) Prominence of blood vessels, normally invisible, best seen in structures, *e.g.*, ear of a rabbit. (3) Perceptible pulsation of fine arterioles and capillaries. (4) Rise of temperature. (5) Swelling of the part which becomes tense.

II. *Evidence of active hyperæmia on the dead body.*—The appearance in the dead body gives us very little information as regards the presence of the hyperæmia during life, except perhaps the prominence of the blood vessels, normally invisible, still persisting after death.

Morbid Physiology.—(1) Swelling of the tissue, but not œdema. (2) Hæmorrhage.—This takes place only when the vessels are already diseased, *e.g.*, in arteriosclerosis. (3) Proliferation of the tissue elements. (4) Disturbance of function. This takes place only in organs which on account of their position cannot expand, *e.g.*, in brain.

PASSIVE HYPERÆMIA OR VENOUS HYPERÆMIA

Etiology.—*Obstruction to venous flow due to*—(1) Causes in the lumen, *e.g.*, thrombosis. (2) Causes in the wall, *e.g.*, phlebitis. (3) Causes outside, *e.g.*, pressure. (4) Gravity, *e.g.*, hypostatic congestion of the lungs. (5) Vasomotor disturbances. (6) Chronic valvular diseases of the heart leading to the engorgement of the systemic circulation. (7) Cirrhosis of the liver leading to the engorgement of the portal circulation.

Morbid Anatomy.—*In the living*—Dark bluish-red colour, lowering of temperature, increased tension of the tissue, swelling. *In the dead*—Bluish-red colour, increased tension of the tissue, the organs are enlarged and the consistency increased, increased quantity of blood in the tissue of the affected organs.

In case of large veins, they become tortuous and dilated and are called *varicose veins.* In smaller veins the part is œdematous and is of light bluish colour. In case of chronic process there is proliferation of fibrous tissue and atrophy of parenchymatous cells. This is best illustrated in the so-called *cardiac liver* or the *nutmeg liver.*

This is a condition of chronic venous congestion in the liver due to chronic valvular affection of the heart. On account of stasis of blood in the inferior vena cava the hepatic veins cannot empty themselves and so they in their turn become engorged along with their tributaries in the liver—the central veins of the lobules.

Fig. 275. Nutmeg Liver.

Morbid Histology.—The central part of the lobule becomes engorged, giving it a reddish appearance, surrounded by the liver tissue which is brownish in colour, thus giving a mottled appearance and hence termed the " nutmeg liver." The central vein in case of a nutmeg liver is found to be dilated along with the tributaries in the central zone of the lobule. The liver cells in this region show evidence of atrophy, due to the pressure of the stagnant blood. Proliferation of fibrous tissue finally occurs, the so-called " cyanotic induration." The same changes take place in the kidneys, lungs and other organs.

Morbid Physiology.—(1) *Changes of the Circulation.*—Slowing of the circulation and rise of the intravenous and intracapillary pressure with dilatation of the same and reflex contraction of the arteries. (2) *Changes of the Circulatory Apparatus.*—Fatty changes of the intima. (3) *Changes of the tissue.*—This depends on the duration of the process and the accommodation of the collateral circulation. This may be (*i*) Changes of the venous or capillary walls;

(*ii*) Hæmorrhage; (*iii*) Œdema; (*iv*) Stasis.—This is of higher degree than ordinary hyperæmia. It is the complete cessation of the circulation, at the same time, there being no coagulation of the blood in the blood vessels; (*v*) Atrophy.—Specially in those organs where the cells are directly affected by pressure, *e.g.*, liver or spleen or in sensitive organs, *e.g.*, kidneys or retina; (*vi*) Cyanotic induration.—This is really not the proliferation of the fibrous tissue but swelling and solidification of the connective tissue fibres, *e.g.*, in liver, lung in mitral disease.

Fig. 276. Nutmeg Liver

Passive Hyperæmia in some organs.—In the *Liver*, the hyperæmia affects generally the central part of the lobule. The central vein dilates with dilatation of the associated capillaries. Hence, the central zone is constituted by dilated capillaries and atrophied liver cells which may ultimately disappear. The portal zone may be normal but often show fatty changes. The appearance of the liver, in advanced stages, is characteristic. Dark red or yellow or yellowish-brown areas alternate, giving a mottled appearance commonly called the "nutmeg liver."

In the *Lung* the interalveolar capillaries are distended and very often the red blood cells escape into the alveolus and either coughed up or disintegrated, the hæmoglobin is converted into hæmosiderin and other brown pigments, which, again, in their turn, are taken up by phagocytic cells which accumulate in the alveolus and are often called "heart failure cells." In chronic condition, the organ, becomes firm, due to cyanotic induration and becomes somewhat brown in appearance. This condition is called "brown induration of the lung."

LOCAL ANÆMIA

Etiology.—I. *Direct.*—Without the influence of the vasomotor system. (*a*) *Compression anæmia.*—Pressure from outside; (*b*) *Obstruction anæmia.*—Due to changes of the vessels, *e.g.*, arteriosclerosis, endarteritis obliterans, etc., or by the presence of thrombosis or embolism.

Intermittent claudication is a condition, generally affecting the leg of horse, when the narrowing of the arteries allow only insufficient amount of blood-supply to the muscles, so that the nutrition is more or less perfectly maintained when the limb is at rest, but walking or running for only a short distance will lead to insufficient nutrition and cramp-like pains.

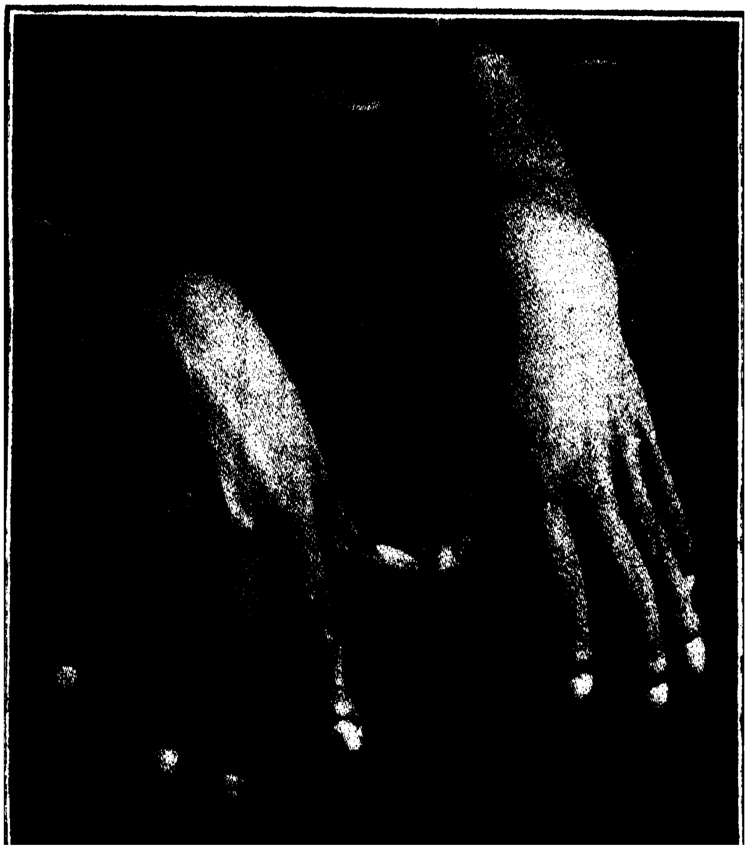

Fig. 277. Chronic hypertrophic (Pulmonary) osteoarthropathy (From Dr. H. Gangulv).

II. *Indirect or neurotic.*—Due to the influence of the vasomotor system. (*a*) *Neurotonic.*—Cold (chilblains), Raynaud's disease, etc.; (*b*) *Reflexive.*—Fear, fainting paleness.

Morbid Anatomy.—I. *In the living*—(*i*) Pallor, (*ii*) Absence of prominence of the blood vessels, (*iii*) Reduction of temperature, (*iv*) Disturbance of function—sometimes paralytic and sometimes irritative manifestations. II. *In the dead body*—(*i*) Pallor, (*ii*) Absence of the prominence of the blood vessels,

Morbid Physiology.—This depends on the (*i*) Duration of the affection, (*ii*) Sensitiveness of the affected organ, (*iii*) Extent of the affected area, (*iv*) Rapidity and perfection of the collateral circulation involved.

The collateral circulation depends on—(*i*) Relation between the affected artery and its branches; (*ii*) Number and size of the communication between the anæmic area and the collateral branches; (*iii*) Extension of the collateral branches and capillaries; (*iv*) Force of the heart.

The result is as follows:—The effects vary according to the period of duration of the circulatory disturbances but mostly depend on the sensitiveness of the tissue affected. The parenchyma is affected early, according to the grade of function, whereas the connective tissue can resist to a considerable extent.

(*a*) After partial stoppage of circulation.—Slowing of circulation, deficiency of nutrition, atrophy. (*b*) After full stoppage of circulation.—All the grades of the retrogressive changes of the cells up to necrosis. (*c*) After sudden stoppage of individual branches of a terminal artery.—Infarction.

HÆMORRHAGE

Etiology.

I. Diapedesis.—For details see Inflammation.

II. Rupture. This is due to (1) Traumatic causes. (2) Disease of the vessel wall—(*i*) Infective or infecto-toxic causes *e.g.*, sepsis. The so-called hæmorrhagic diathesis is mostly infectious in nature. (*ii*) Toxic—(*a*) Endogenous.—In chronic nephritis (changes in the vessel wall in addition to the rise of blood pressure), leukæmia, oligocythæmia, chronic jaundice, scurvy, Barlow's disease, etc.; (*b*) Exogenous.—Acute phosphorus poisoning, chronic morphinism, mercury poisoning, snake venom or CO_2 poisoning. (3) Increased pressure within the vessels—(*i*) Congestive—This is illustrated by death by drowning or hanging, where hæmorrhage takes place in serous sacs, the so-called Tardius spots. This is also found is cases of diphtheria and may be absent in many cases of drowning; (*ii*) Obstruction of the blood vessels.—This is due to the obstruction of small vessels and capillaries, as by hyaline thrombi in eclampsia or fat embolus in the lung or brain; (*iii*) *Nervous.*—In hysteria, menstruation, etc.

Varieties of Hæmorrhage.—(1) Petechia—minute hæmorrhages under the skin or mucous membranes; (2) Suffusion, suggilation or extravasation—same as above but very large and diffuse; (3) Hæmatoma—accumulation of blood in the form of a tumour; (4) Epistaxis—bleeding from the nose; (5) Hæmoptysis—bleeding from the lungs; (6) Hæmatemesis—bleeding from the stomach; (7) Melæna—bleeding from the intestines; (8) Piles or hæmorrhoids —bleeding from the rectum; (9) Menorrhagia—bleeding from the uterus during the menstrual periods; (10) Metrorrhagia—bleeding from the uterus between the menstrual periods; (11) Hæmatocele—bleeding into the tunica vaginalis; (12) Hæmothorax—bleeding into the pleural cavity; (13) Hæmopericardium—bleeding into the pericardium; (14) Apoplexy—bleeding into the brain.

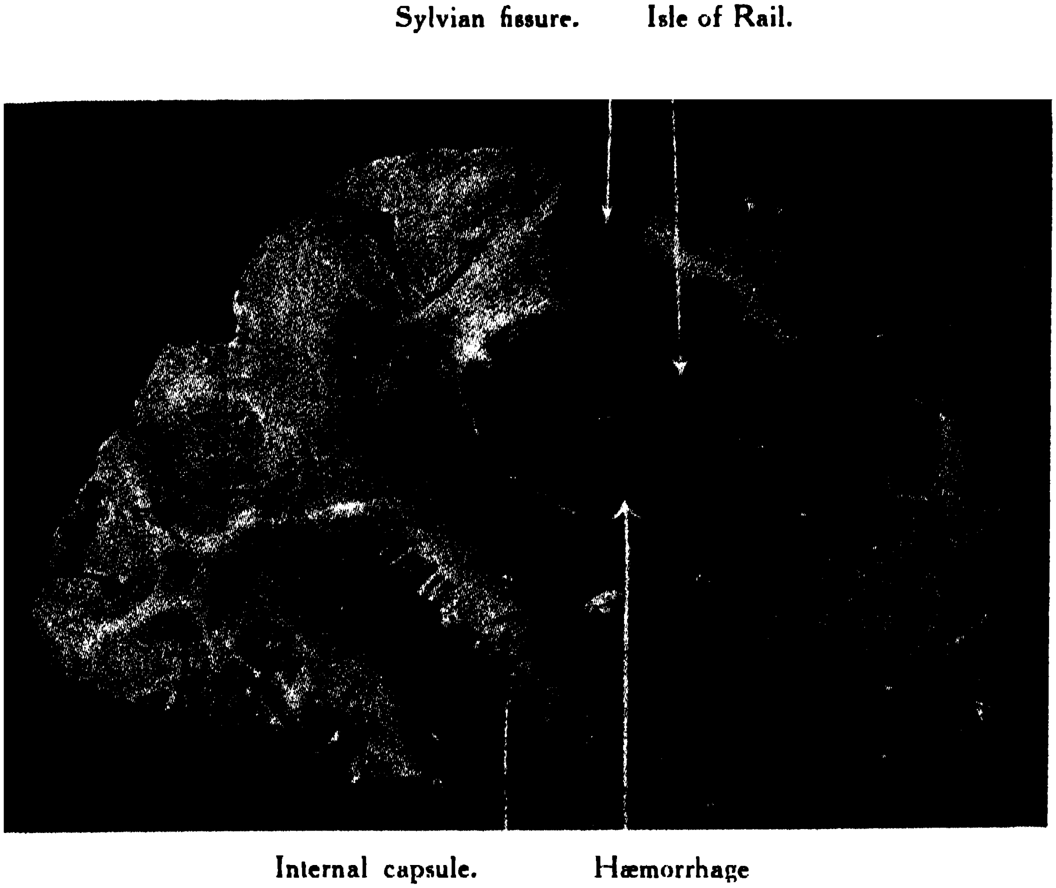

Fig. 278. Total frozen section of brain showing hæmorrhage in the external capsule.

Result of Hæmorrhage.—(1) For the circulation—fall of blood pressure and so spontaneous stoppage of hæmorrhage; (2) For the affected organ—see colliquation necrosis; (3) For the whole body—chronic anæmia.

ŒDEMA

Œdema is the accumulation of watery fluid from the vessels into the tissue interstices or in closed cavities.

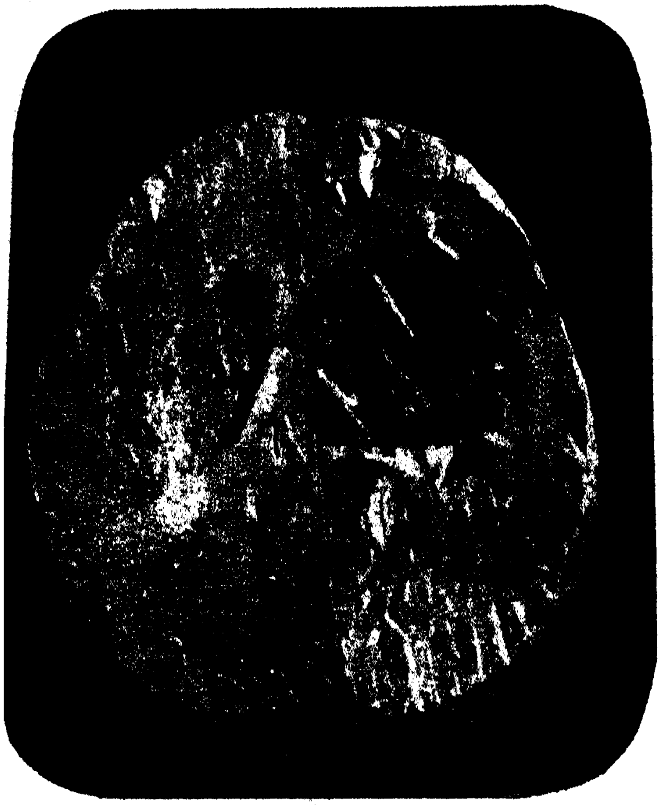

Fig. 279. Cerebral hæmorrhage.

Theories as regards the Origin of Tissue Fluids.—(1) *Filtration* theory (Ludwig).—This takes place, when the blood pressure is greater than the resistance of the tissue, leading to an outflow of fluid through the vessels. Conversely, when the tissue pressure is greater than the blood or lymph pressure, the fluid elements of the tissue enter into the vascular system. This takes place in the so-called resorption or absorption of fluid from the tissues. (2) *Diffusion Transudation* theory (Cohnstein).—This depends on the transference of fluid elements due to the difference in the osmotic pressure on the two sides of the capillary wall. (3) *Secretion* theory (Heidenhein).—This depends on some vital process of the cells.

Etiology.—1. *Active.*—Due to rise of endocapillary pressure. Experimental finding:—much œdema of the tongue follows after irritation of the lingual nerve in dogs. Section of N. ischiaticus is followed by œdema only when the femoral vein of the same side is also ligated. In human pathology this condition is exemplified by hemi-anasarca.

Quinck's disease. This condition is difficult to explain. Von Recklinghausen regarded it to be a collateral inflammatory process. Lubarsch is of opinion that this is due to injury of the capillary walls by micro-organisms and their toxins.

Fig. 280. Small-pox pustule showing inflammatory œdema.

The œdema of acute nephritis has been explained by the fact that the anuria causes a serous plethora and rise of blood pressure, but infusion of a large quantity of normal saline in animals is rarely followed by œdema.

2. *Due to diminution of tissue pressure.*—This is exemplified by œdema *ex vacuo.* Œdema of pia mater occurs in cases of atrophy of the brain. But all pia mater œdema is not *ex vacuo.*

3. *Abnormal permeability of the vessels.*—This takes place in infectious processes, *e.g.*, in malignant œdema, anthrax, etc. Urticaria is also an inflammatory œdema. Cachectic œdema, inflammatory œdema of the lungs are also examples.

4. *Change of the osmotic pressure.*—In chronic nephritis there follows a hyper-albuminous condition and hence œdema. Here also the injury of the blood vessels plays a great part.

5. Rise of osmotic pressure of the tissue fluid. } Of no
6. Increased secretion .... } importance.

Varieties of Œdema.

1. *Mechanical.*—This takes place when there is some obstruction to the circulation leading to increased intravascular pressure. The circulation affected is most commonly either the venous or the lymphatic. (*i*) *Obstruction to the venous circulation.*—Bier's hyperæmia, ascites due to obstruction of the portal vein, are examples of venous obstruction. Simple ligature of the femoral vein does not produce any œdema, unless the N. ischiaticus is also cut simultaneously. The purest form of passive œdema is found in cases of lung, so long as no nervous influence or inflammatory process is present. In cases

of passive œdema, the condition of the arteries too plays a very important part, *e.g.*, in the so-called angiosclerotic œdema. Thomas experimented on dead bodies and found that with normal vessels it took 17 litres of normal saline to produce œdema, whereas only 4 litres were sufficient in cases with atheromatous vessels. (*ii*) *Obstruction to the lymphatic circulation.*—Obstruction of the lymphatic channels (ductus thoracious) by tumours leads to chylous ascites, lymphorrhagia, etc. Ligature of the thoracic duct in horse produces no œdema (Dupuytren). Hydrops chylous should be differentiated from hydrops adiposus. The former is lymphorrhagia, due to bursting of the lymph vessels, whereas the latter is a mixture of fat-laden endothelial or tumour cells (cancer) with the serous fluids in the sac.

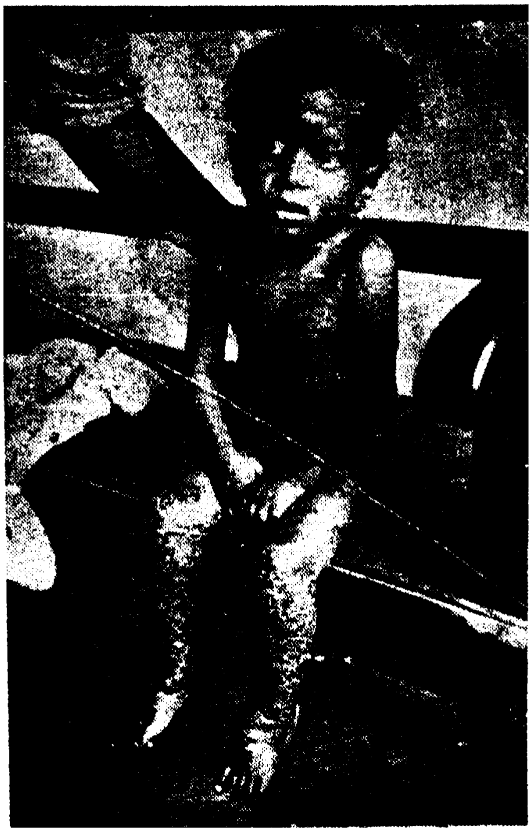

Fig. 281. Eczematoid type of Seborrhœa. (From Cal. Med. Journal).

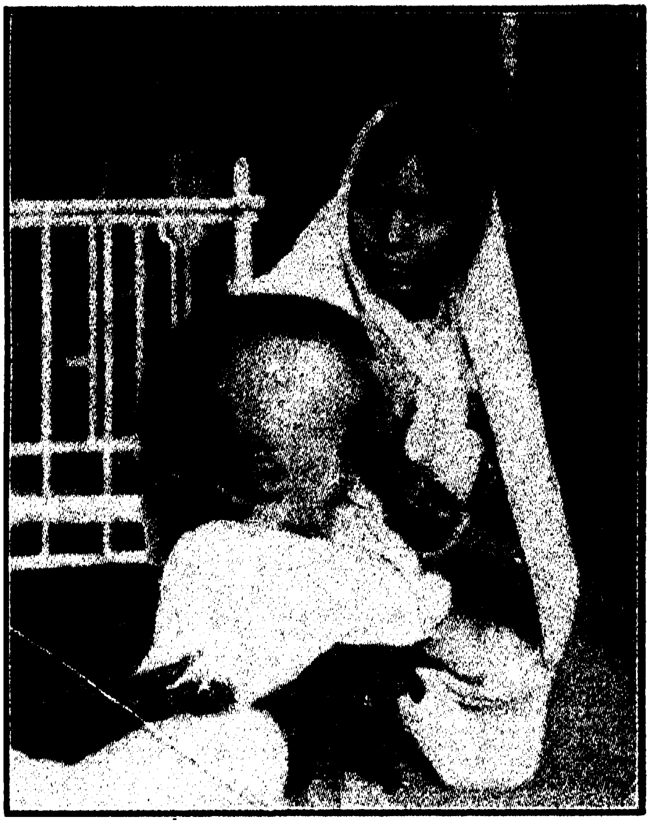

Fig. 282. Hydrocephalus. (From Dr. P. K. Mitra).

Œdema of lungs is commonly an agonal condition. This may be due to (*i*) Œdema of passive congestion, (*ii*) Œdema of inflammation, (*iii*) Œdema of nervous origin. Œdema of lungs may be acute and may lead to sudden death.

2. *Neurotic.*—This is exemplified in case of angioneurotic œdema, due to the disturbance in the vaso motor system, either irritation of the vasodilators or paralysis of the vaso constrictors.

3. *Chemical.*—This depends on the action of chemical substances on the capillary wall, altering thereby the permeability through them. The commonest examples of these chemicals are (*i*) *Direct toxic œdema.*—Bacterial toxin, *e.g.*, diphtheria, rheumatic joints, etc., bites of insects; urticaria after taking some particular food; (*ii*) *Indirect toxic œdema.*—This includes the influence of toxins on the kidneys, leading to nephritis and œdema; (*iii*) *Nutritional disturbances.*—The so-called cachectic œdema.

4. *Inflammatory.*—This depends on inflammatory processes, resulting from action of toxins, leading to injury to the blood vessels and tissues.

Difference between Transudation and Exudation

Transudate.	*Exudate.*
Poor in albumen (0 07%-0 5%)	Rich in albumen (more than 4%)
No spontaneous coagulation	Spontaneous coagulation.
Specific gravity—1,006-1012	Sp. gr. 1018-1020.
Clear with no fibrin flakes	Turbid and rich in fibrin flakes.
Serous surface—shining and smooth.	Dull, injected or fibrin deposition.

N. B.—Normally about 15-30 c.c. of fluid is found in pericardium and also in the dependent part of pleura and peritoneum. This quantity increases if the body is examined long after death or putrefaction has commenced.

Result of Œdema.—(1) *Degenerative Changes.*—This is mainly due to pressure and the cells undergo hydropic-vacuolar degeneration, the connective tissue fibres being swollen up. (2) *Regenerative changes.*—This is exemplified by cases of chronic œdema of lungs, the so-called splenisation of the lung, on account of, its resemblence to spleen in colour and consistency. (3) *Inflammatory changes.*—Due to access of micro-organisms or autotoxic substances.

STASIS

Stasis is the condition in which there is cessation of blood flow, but still retaining all the normal constituents and without any coagulation taking place.

Etiology.—1. This is chiefly due to the loss of fluid elements from blood, either through absorption of fluids from the blood, or, through damaged vessel-wall or increased pressure. 2. *Thermal* (by damaging the vascular endothelium).—This takes place when the temperature is very low (—7°C.) or very high (50°C.). 3. *Chemical.*—This takes place through substances which withdraw fluids either directly or indirectly, *e.g.*, sugar, glycerin, nitrates, urea, etc., or, which produce damage to the vessels, *e.g.*, ether, chloroform, etc.

Morbid Anatomy.—The contents of the vessel look like homogeneous dark-red non-moving pillar of blood.

Pathological Physiology.—1. *For the circulation*—(*i*) Hæmorrhage by diapedesis. (*ii*) Leucocytes migrate towards the wall of the vessel. (*iii*) Wandering of leucocytes and fluid contents. 2. *For the tissue.*—The changes are similar to those of anæmia, *i.e.*, atrophy, necrosis or infarction.

THROMBOSIS

Thrombosis is the intravascular or intracardiac coagulation of blood during life. This is differentiated from a clot or coagulum which takes place in the blood outside the blood vessels or after death.

Etiology.—1. Changes in the quantity and quality of blood. 2. Changes in the rate of flow of the blood—slowing of circulation. 3. Changes in the inner wall of the blood vessels—(*a*) Roughening of the intima, *e.g.*, in arteriosclerosis and aneurism. (*b*) Rupture of the intima due to ligature of the vessel. (*c*) Inflammation of the vessel wall.—Angitis (inflammation of a vessel) is always followed by thrombosis and *vice versa.*

Infection plays a great part in the formation of thrombosis. Thrombosis of veins is nearly always due to infection. Lubarsch found bacteria by culture in about 60 per cent. of apparently aseptic thrombus.

The micro-organisms act in the following ways:—(*a*) On the blood—by hæmolytic influence. (*b*) On the heart and the vasomotor mechanism—by slowing the rate of flow. (*c*) On the vessel wall—(of lesser importance).

Seat of Occurrence.—Thrombosis may occur in any part of the vascular system, the heart, arteries, veins or the capillaries. 1. *Cardiac thrombosis.* In the auricles, the appendix is commonly affected, in cases of distension of the auricles, by cardiac valvular diseases. The thrombus may be of red appearance and is known as the "ball thrombus." In the ventricles, thrombosis generally takes place in the apex of the left ventricle. The valves are also the commonest sites for thrombosis, the early stages of endocarditic vegetations are essentially nothing but thrombi. 2. *Arterial thrombosis.*—This generally affects the smaller arteries, where the blood stream is rather slow. The rate of flow and the condition of the vessel wall play the most important part. In the aorta, extensive roughening of the inner surface may be associated with no thrombosis, but in the case of aneurism, some amount of laminated thrombus is invariably present, often filling up the whole cavity. 3. *Venous thrombosis.*—Veins are affected much oftener than the arteries, although the roughening of the lining surface occurs more often in the arteries. The slowness of the circulation is mainly responsible for the thrombosis in the veins, especially when this is associated with any inflammatory condition. This occurs in cases of typhoid, in puerperium or in marasmatic conditions. 4. *Capillary thrombosis.*—This occurs in cases of inflammation.

Forms of Thrombosis.—1. *Red thrombus.*—This is formed when the process of coagulation is very rapid. This consists of red blood cells, fibrin, and very few blood platelets. 2. *White thrombus.*—This is characterised by its architectural peculiarity. The structure is formed of coral-like foundation of blood platelets on which leucocytes and fibrin are arranged in various quantities and forms. 3. *Mixed or laminated.*—This is similar to the white variety, only various amount of red blood cells is also present. This appears as chicken fat, the dependent parts being red and superficial parts white. This takes place inside the heart, where the intact endothelium keeps the blood sufficiently long, without clotting. In the meantime, the red corpuscles, being heavier than the plasma, settle at the bottom with the leucocytes at the top, and, coagulated plasma, practically free from any cellular elements, remain at the top. 4. *Granular or platelet thrombus.*—There is no special architecture. It is more or less regular, but, on magnification, shows a granular surface and is generally found in the capillaries and small veins. 5. *Hyaline.*—This form is also found in capillaries and small veins, specially of the brain, lungs, kidneys, etc., and produces either a homogeneous filling up of the vessel or a combination of a series of individual round particles. They stain intensely with acid aniline dyes. They are generally found in infectious diseases (*e.g.*, pneumonia, influenza), autointoxication (*e.g.*, eclampsia) or toxic processes (*e.g.*, ergotin).

Varieties.—Clots may be—(1) Ante-mortem—occurring before death. (2) Poro-ante-mortem—occurring during the process of dying. (3) Post-mortem,—occurring after death.

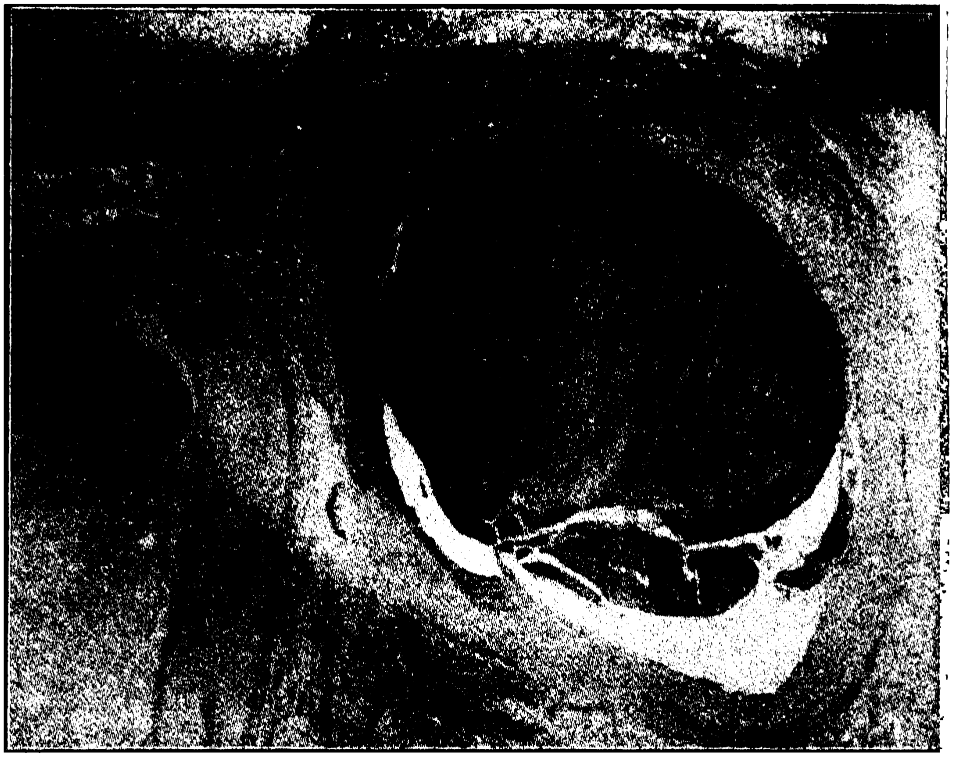

Fig. 283. Thrombosis of vein. The artery is seen by the side of the thrombosed vein.

Poro-ante-mortem clot—appears like chicken-fat, deeper parts being red and superficial parts white.

Difference between *post-mortem* and *ante-mortem* clot:—

Post-mortem clot.	*Ante-mortem clot.*
Dark coloured.	Colour—pale.
Easily friable.	Very tough.
Loosely attached	Adherent to the vessel wall.

Thrombi may be—(*i*) Parietal thrombi—These are thrombi occupying only a part of the wall of the vessels without any obstruction to the flow of blood through the vessels. (*ii*) Ball thrombi—These are parietal thrombi but more rounded like balls and are commonly found in the auricles and other chambers of the heart. (*iii*) Polypoid thrombi—These are ball thrombi with stalks or pedicles. (*iv*) Valvular thrombi.—These are polypoid thrombi, acting inside the heart or the vessels.. (*v*) Occluding or central thrombi—These occur when the whole lumen of the vessels is completely obliterated or occluded. (*vi*) Tunnelled or channelled thrombi—These are due to canalisation of the occluding thrombi. (*vii*) Organised thrombi—These are due to the replacement of the thrombi by fibro-connective tissue, the substances of the thrombi being partly absorbed and partly removed by leucocytes. (*viii*) Softening thrombi—These are due to (*a*) Disintegration of the centre of the thrombi as a retrogressive process and consist of fibrin, blood corpuscles, fat, pigment, etc. (*b*) infection by pyogenic or putrefactive organisms. (*ix*) Calcified thrombi—These are due to the deposition of calcareous particles in the thrombi. These are called *phleboliths* (when occurring in veins), *arterioliths* (when occurring in arteries), etc.

Macroscopical Difference between Thrombus and Clot.—(*a*) The thrombus, even when fresh, is more or less dry, more fixed, more friable and less elastic than clot. (*b*) The thrombus has a surface which is never smooth and glistening, rather finely granular. (*c*) The thrombus shows on section an irregularly laminated appearance, with a mixture of red and colourless areas. (*d*) The clot lies free in the vessel without fixing itself with the wall.

Microscopical Difference between Thrombus and Clot.—(*a*) The clot consists of the blood elements in the same relation as they exist in the circulation. (*b*) The thrombus, often contains those elements of the blood, which are present only sparingly in normal condition (*e.g.*, blood platelets), or not at all (*e.g.*, hyaline either granular or in clumps). Fibrin, the chief constituent of clot may also be absent in thrombus. (*c*) The constituents forming the thrombus, *e.g.*, red blood cells, leucocytes, platelets, etc., show some regular arrangement which often fails in the clot.

Mechanism of the Formation of the Thrombus.—The earliest event is the deposition of a few platelets on the wall of the blood vessel, which increases in size by the addition of more platelets by adhesion. These masses of platelets are soon covered by the circulating leucocytes which stick to them and liberate thromboplastic substances leading to the deposition of fibrin filaments, which, again, in their turn, entangle masses of red corpuscles. Thus a more or less, solid mass of cells and fibrin is formed on the wall of the vessel which appears red with a mixture of greyish-white colour of platelets. This increases in size and according to the age, shows various grades of colour and may be laminated.

Blood platelets play the most important part in the formation of thrombus, generally through their adhesive character, assumed by them when shedding of blood takes place. Hence, the etiological factors of thrombosis act in either of the two ways:—(*a*) Injuring the endothelial lining of the vessel, bringing out the adhesive character of the platelets. (*ii*) Bringing the platelets into contact with the injured endothelial lining.

Results of Thrombosis.—Thrombosis often serves a protective function, as in cases of infective processes, it acts as a barrier to their access into the general circulation, or when a vessel is involved in an ulcerative process, the occurrence of hæmorrhage is prevented, if there be any thrombosis.

The results are as follows:—(*i*) Obstruction to the circulation—this depends on the part of the vessel affected, rapidity of formation, relative size of the vessel and the thrombi and the number of collateral branches. *Phlegmasia alba dolens* is a condition due to thrombosis of the ilio-fermoral vein in the puerperal state, extending to the junction of the external illac vein, forming what is known as the "milk leg." (*ii*) Embolism—this is due to the lodgment of portions of thrombi in different parts of the body, being detached and carried away by the circulation. (*iii*) Inflammation and organisation of the vessel wall with formation of thrombangitis (thrombo-phlebitis, thrombo-arteritis). (*iv*) Œdema. (*v*) Hæmorrhage. (*vi*) Tissue changes. Infection of the thrombus plays a great role in producing consequent additional changes.

EMBOLISM

"*Embolism,*" as defined by McFarland, "is intravascular obstruction from the lodgment of any circulating body." "The obstructing body is the *embolus.*"

Etiological Varieties of Emboli.—1. Portions of thrombus—thrombus-emboli. 2. Emboli of normal, pathologically altered or newly formed tissue, or a fine part thereof. As for instance—(*i*) Gas emboli. (*ii*) Fat emboli. (*iii*) Pigment emboli. (*iv*) Tissue or tissue-cell emboli. (*v*) Tumour or tumour-cell emboli. 3. Exogeneous emboli—*i.e.*, emboli due to foreign bodies:—(*i*) Air emboli. (*ii*) Vegetable parasite. (*iii*) Animal parasites. (*iv*) Any other foreign bodies.

Classification.—According to the seat of occurrence: 1. Typical. 2. Atypical or paradoxical emboli.—These are emboli which take place in the opposite direction to the circulation. They occur in cases of (*a*) Open foramen ovale (being patent in about 25 per cent. of all persons), (*b*) Stasis and increased pressure in the right heart, *e.g.*, in bronchitis, emphysema, chronic tuberculosis, etc.

Thrombus-Emboli and their Identification

These are most common in the pulmonary arteries (about 60 per cent. of all emboli).

Identification.—1. Presence of primary thrombus. 2. The resemblance of the embolus to the thrombus. 3. Presence of irregular, ladder-like broken end of the embolus which fits with the thrombus. 4. Disproportion between the age of the embolus and the relation of the wall. 5. Seat, position, and arrangement of the embolus. 6. Clinical manifestation during life.

Mechanism and Condition of Origin of Thrombus-emboli.—(*a*) Parietal thrombus, especially when it becomes soft and carried away by the blood stream. (*b*) Occluding thrombus, when it is still fresh and not yet firmly attached to the vessel wall. It is exceptional for a thrombus, older than 10 days, to cause embolism.

Results of Thrombus-Emboli.—1. Effects on the vessel. These are same as the primary thrombus, *e.g.*, stasis of blood, œdema, hæmorrhage and growth of tissue but, when the thrombus is an infected one, similar process extends to the wall also. 2. Effects on the circulation.—These are the same as those of thrombus. 3. Effects on the tissue of the organ affected.—This depends on the nature of the vessel affected and the extent of the obstruction. As for example, —sudden death occurs in embolism of the coronary artery or sudden blindness in case of the retinal artery, Similar serious symptoms occur in embolism of the brain, lungs, etc. 4. *The most inportant effect of embolism is Infarction.*

INFARCTION

An *Infarct* is a tissue area whose nutrition is prevented by embolic obstruction of its "end-artery." The term "end-arteries" as given by Cohnheim means that those arteries possess no, or, only imperfect anastomosis and are insufficient to keep the part alive in case of their obstruction.

Infarction is the process of the formation of an infarct. Infarctions are of two main types—(1) Pale or Anæmic. (2) Red or hæmorrhagic.

Some Important Facts regarding the Blood Supply of Different Organs with Reference to Infarct Formation

Kidney.—Outer layer of the cortex receives its blood supply from the vessels extending to it from the capsule. Hence a layer of living, often hyperæmic, tissue covers the infarct towards the surface.

Spleen.—No such circulation from the capsule, hence the infarct extends up to the peritoneal surface.

Lungs.—Bronchial arteries are the main nutrient arteries of the lungs, specially the bronchi and the lung tissue immediately surrounding it. Pulmonary artery anastomoses freely with wide capillaries.

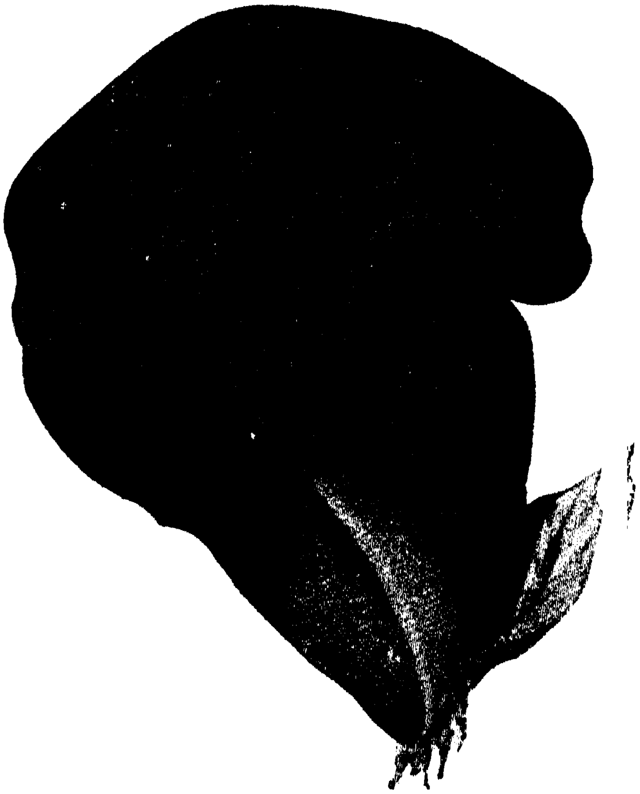

Fig. 284. Anæmic infarct of the spleen. (Path. Museum, Carmich. Med. Col.)

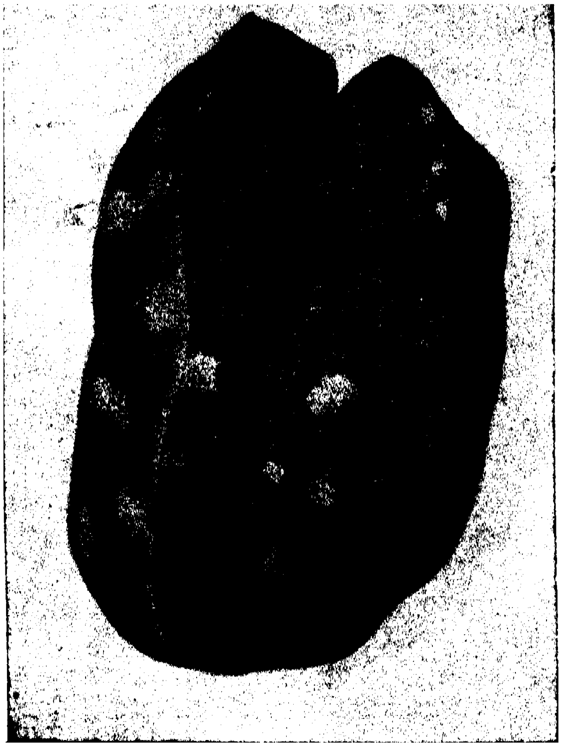

Fig. 285. Multiple infarct spleen. (From Path. Museum, Cal. Med. Col.)

Liver.—Infarcts are very rare on account of extremely rich blood supply from two sources—the hepatic artery and the portal vein.

Retina.—The central artery is an end-artery.

Large branches of the coronary arteries, arteries of the basal ganglia of the brain, superior mesenteric artery are also end-arteries.

Macroscopical Characters of Anæmic Infarct.—The effect of infarction is always necrosis of the tissue affected. Usually it is of coagulative type, but in case of the brain, softening is the prominent feature (the so-called colliquative necrosis).

The infarct is (1) Wedge-shaped, pale area. (2) Raised above the surface. (3) Margin—red. (4) Cut surface—slightly granular.

Microscopical Characters.—The cells are swollen with faintly stainable or unstainable nuclei. The cells are all dead and are entangled in a coagulum produced by the coagulation of the fluids diffused into the area from the surrounding tissue. This process is called " coagulative necrosis " by Cohnheim. The margin of the infarct is hyperæmic and full of leucocytes loaded with fat cells and glycogen.

Conditions for the Origin of the Anæmic Infarct.—1. Rapidity of the obstruction. 2. Sensitiveness, or, any other special condition, of the tissue before the obstruction. 3. Power of the heart 4. Extension of the existing anastomosis. 5. Number and size of the collateral circulation. 6. Size of the obstructed vessel.

Seat of Occurrence of the Anæmic Infarct.—(*a*) *According to the frequency of occurrence.*—Kidney, spleen, brain, extremities, heart, retina, spinal cord, intestine, stomach, and testes. (*b*) *Never occurs in*—Urinary bladder, external and internal genital organs, thyroid, tongue, adrenals, bone and hypophysis.

Termination of Infarct.—This depends mainly on the nature of the tissue affected and the size of the affected part. It is found that the resorptive processes appear rapidly and the degenerated elements are rapidly transported away, leaving spaces to be filled up by connective tissue. In hæmorrhagic infarcts some amount of blood pigment remains for a considerably long time.

The termination is as follows:—(1) Non-bacterial softening (colliquative necrosis) takes place in case of the central nervous system. (2) Organisation results in a scar. (3) Septic processes when the causative embolus is a septic one.

Hæmorrhagic Infarct

Morbid Anatomy.—The affected part is soft, wedge-shaped and is dark red in colour. Microscopically, all the meshes of the tissue are full of red blood cells. The cellular elements may show no visible changes but are often necrotic and show deficient nuclear staining.

Conditions for the Origin of Hæmorrhagic Infarct

1. Hæmorrhagic infarct occurs when the capillary stream is specially strong as in case of the lung and the spleen which are normally rich in blood, and the blood vessels are wide and more dilatable and yielding, on account of soft parenchyma.

2. When the capillaries possess a special permeability, either normally or due to any diseased process. The lungs, spleen and intestines possess very thin capillaries. For this reason, there is never any anæmic infarct in the lungs, more specially because infarction usually takes place, not in normal lung, but in one which is already congested and thereby the capillaries are extremely permeable.

3. When the blood in the obstructed area contains some substance which leads to injury to the vessel wall. In the intestine the presence of bacteria, producing increased permeability of the capillary wall, forms a very important factor.

Seat of Occurrence of Hæmorrhagic Infarct.—*According to frequency.*—Lungs (never anæmic infarct), spleen, skin, intestine. *Rarely occurs in*—Heart, brain and liver and extremely rare in the kidneys.

OTHER FORMS OF EMBOLI

Gas Emboli.—Emboli caused by free nitrogen in blood, *e.g.*, in caissons disease.

Result—This depends on the size of the gas bubble. (1) By small bubble.—Obstruction of the capillaries, specially in case of the brain, resulting in softening. (2) By big bubble.—Disturbances of the activity of the heart and the lungs.

Fat Emboli.—Emboli caused by fluid fat in the organs.

Etiology.—(1) Injury or pathological disorders of the bone marrow, subcutaneous fat or other bigger fatty depots of the body, *e.g.*, fracture, laparotomy of fatty persons, acute osteomyelitis, etc. (2) Severe shaking of the bones, or, of the whole body, *e.g.*, in eclampsia, epilepsy, etc. (3) Resorption of big fat masses by the lymphatics.—This is the same as the others, only, lymphogenous, instead of hæmatogenous.

Seat of Occurrence.—Chiefly localised in the pulmonary circulation. Systemic circulation may also be affected in case of open foramen ovale or through the pulmonary capillaries by strong contraction of the heart.

Result.—This depends on the nature of the obstruction and the activity of the heart. This also depends on the organ affected. In case of the lungs it is not dangerous if the heart is good. It produces only some amount of hæmorrhage, œdema and hæmorrhagic infarction. In brain and the heart it is much more dangerous.

Fate of the fat embolus.—(1) Part is dissolved and removed by the fluids of the body. (2) Part is disposed of by the activity of the cells. Generally the fat embolus disappears within 14 days.

Pigment Emboli.—Obstruction of the blood or lymph vessels by lodgment of pigment derived from the body, or set free from pre-existent pigment mass. This is very rare.

Cell of Tissue Emboli.—These are cells or tissue originating from (1) Placenta. (2) Bone marrow. (3) Fat.

Etiology.—(1) Traumatic (eclampsia). (2) Infection. (3) Toxic.

Tumour cell or Tissue Emboli.—These play a very important part in pathology, specially, in cases of carcinoma, sarcoma, choronepithelioma, melanocytoblastoma, etc. It must be remembered that all embolism of tumour tissue does not regularly lead to metastasis, because many of the cells degenerate.

Air Emboli.—Obstruction, by the introduction of atmospheric air into the circulation, *e.g.*, opening of the veins during injection, operation in the region of the neck, erosion of the gastric veins by gastric ulcer, obstetrical operation, etc. Animals, especially dogs, can bear 5 to 6 times the amount of air that man can bear, without any disturbance.

Bacterial Emboli.—This consists of obstruction of the lymph or blood channels not by single organism but by a mass of the same.

Etiology.—It is caused most commonly by septic processes, resulting in septic thrombus in veins or arteries, which serves as the source of the masses of the organisms that cause embolism. This is commonest in septic endocarditis, where pure culture of staphylococcus or streptococcus exists on the valves and is detached, to be carried away in the circulation. Septic osteomyelitis is also a common factor.

Result.—A secondary abscess results from such embolus, but not always. Emboli of streptococcus or staphylococcus may produce only simple hæmorrhage and tissue necrosis without any abscess formation.

Emboli of Animal Parasites.—*Obstruction* in the blood vessels by animal parasites, *e.g.*, in cerebral malaria. This does not include instances where obstruction takes place by animal parasites *distributed through* the blood or lymph vessels, *e.g.*, trichina spiralis from the intestines.

METASTASIS

Metastasis is the appearance of new foci of the affection exactly similar to the primary one.

Difference between Metastasis and Emboli.—The metastatic process most commonly takes place along the route of emboli but not always. The metastasis is characterised by the property of spreading and inoculating the germ.

Varieties of Metastasis.—1. Parasitic. 2. Tumour.

Parasitic Metastasis

The vegetable parasites are responsible for this, whereas the animal parasites play very little part, except the echinococcus. The severity of the affection depends on the number of the organisms transmitted.

Methods.—(1) Obstruction by individual bacteria through blood or lymph channels, *e.g.*, gonorrhœal arthritis, pneumococcal meningitis in cases of acute lobar pneumonia, affection of the lymph glands in plague etc. (2) Obstruction, through bacterial emboli, of the capillaries, *e.g.*, miliary abscess of the kidneys in ulcerative endocarditis. (3) Obstruction through direct inoculation, *e.g.*, tuberculous laryngitis or tuberculous ulcer of the intestine in cases of chronic pulmonary tuberculosis.

Tumour Metastasis

This results from the obstruction of the blood or lymph channels by the tumour cells.

Methods.—(1) By lymphatic channels—This takes place chiefly in cancer (2) By blood channels—*e.g.*, chiefly in sarcoma, chorion-epithelioma, etc. (3) By inoculation—*e.g.*, metastasis in the pouch of Douglas or omentum in cases of gastric cancer. Carcinoma on the points, just opposite the lips or vulva, etc.

Conditions of Tumour Metastasis and its Special Localisation

This depends on—(1) The size of the obstructing tumour-cell or tumour mass. (2) Direction of circulation and distance of the affected organ. (3) Biological relations either chemical, nervous or physiological. This is illustrated by the special affinity of carcinoma of the thyroid, breast or the prostate to have metastasis in the skeletal system. This depends on the physiological relations between the functions of the thyroid and sexual organs to the skeletal system.

CHAPTER XXXII.

DISTURBANCES OF NUTRITION AND METABOLISM

The disturbances of nutrition and metabolism generally lead to retrogressive processes which may vary greatly in intensity from simple atrophy to the other extreme, *i.e.*, the death of the cell or what is known as necrosis.

The metabolic processes of the body proceed normally on some definite relation to the different cellular elements or the organism as a whole. This equilibrium may be altered by the diseased processes and changes take place either in the individual cells or organs or the whole body according to the different amount and nature of the etiological factor concerned. In the same way the whole framework of the body may be uniformly enlarged or reduced and is known as hypertrophy or atrophy respectively. This may also happen in connection with each individual cell or organ of the body.

The disturbances of nutrition and metabolism can be divided into (1) Atrophy; (2) Disturbance of the protein metabolism; (3) Disturbance of the fat metabolism; (4) Disturbance of the carbohydrate metabolism; (5) Disturbance of the mineral metabolism; (6) Disturbance of the pigment metabolism; (7) Necrosis and gangrene.

ATROPHY

This is a condition in which there is wasting or diminution in the size of a tissue or its component elements, due to the diminution of its specific tissue elements.

Emaciation.—It is the condition in which there is wasting of the whole body. Atrophy should be differentiated from the following conditions.—*Hypoplasia* (numerical atrophy).—Consisting of a smaller number of the specific tissue elements. *Aplasia.*—Absence of development.

Etiology.—Virchow classified this as passive and active. Now it is classified as follows (Lubarsch):—

1. *Exogenous* (nutritive or passive atrophy of Virchow).—This condition arises when the cells get a deficient amount of nutrition, *e.g.*, in inanition or long continued hunger. The brain, bone and suprarenal rarely show loss of weight due to nutritional disturbances. The fat

depots of the body and skeletal muscles are mostly affected. Spleen, liver, and kidney also lose weight.

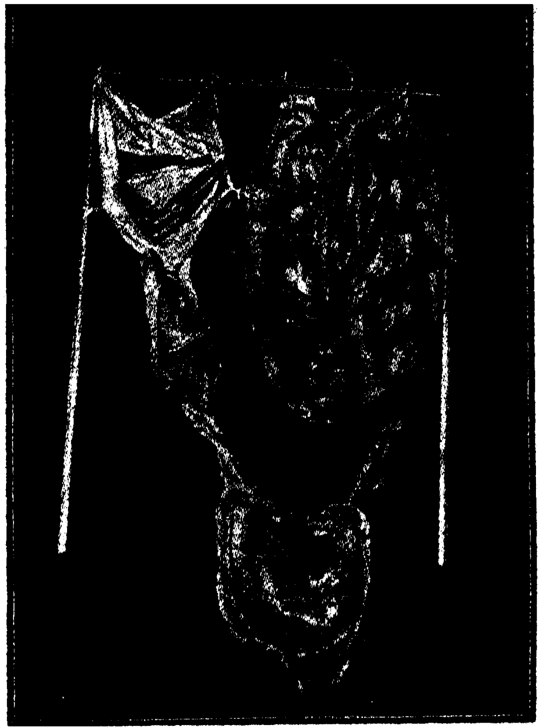

Fig. 286. Hydronephrosis.

2. *Endogenous or cellular atrophy.*—This may be due to (A) *Direct causes.* (*a*) Disuse or diminution of function.*—Disappearance of the tail of a tadpole, of the thymus and other structures in man and atrophy of the muscles of the limbs in chronic diseases of joints are examples. (*b*) Senile atrophy (physiological atrophy).—This generally takes place in the bones and ovaries, *e.g.*, in climacterium.

(B) *Indirect causes.*—(*a*) Mechanical. (*i*) Pressure.—This must be continuous and uniform. It is illustrated in the deformed foot of the Chinese lady (*see* Fig. 18) and in atrophy of the liver cells by pressure of fibrous tissue in cirrhosis of liver, or by atrophy of the kidney by hydronephrosis, vertebræ by aortic aneurism, flattening and, ultimately, loss of epithelial lining of glands or their ducts, in cystic dilatation. (*ii*) Vascular disturbances, *e.g.*, thrombosis, disease of the vessel wall or pressure on the vessel from outside.

(*b*) Trophic changes (trophoneurosis), *e.g.*, neuritis, progressive muscular atrophy, leprosy, syringomyelia, etc. This is regarded by some to be due to (1) Loss of sensibility. (2) Vasomotor disturbances.

Morbid Anatomy.—When the affected structure is superficial to the surface of the body or body cavities, it is very easily detected by numerous folds on the surface. In atrophy of the whole organ it looks smaller and the blood vessels show increased tortuosity.

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* Lubrasch thinks that disuse atrophy is caused by autotoxic causes due to defective removal of metabolic products.

Morbid Physiology.—There is generally deficiency in the function of the affected organ. In case of the heart and liver it is often found that in spite of extensive brown atrophy there is very little marked qualitative functional disturbance.

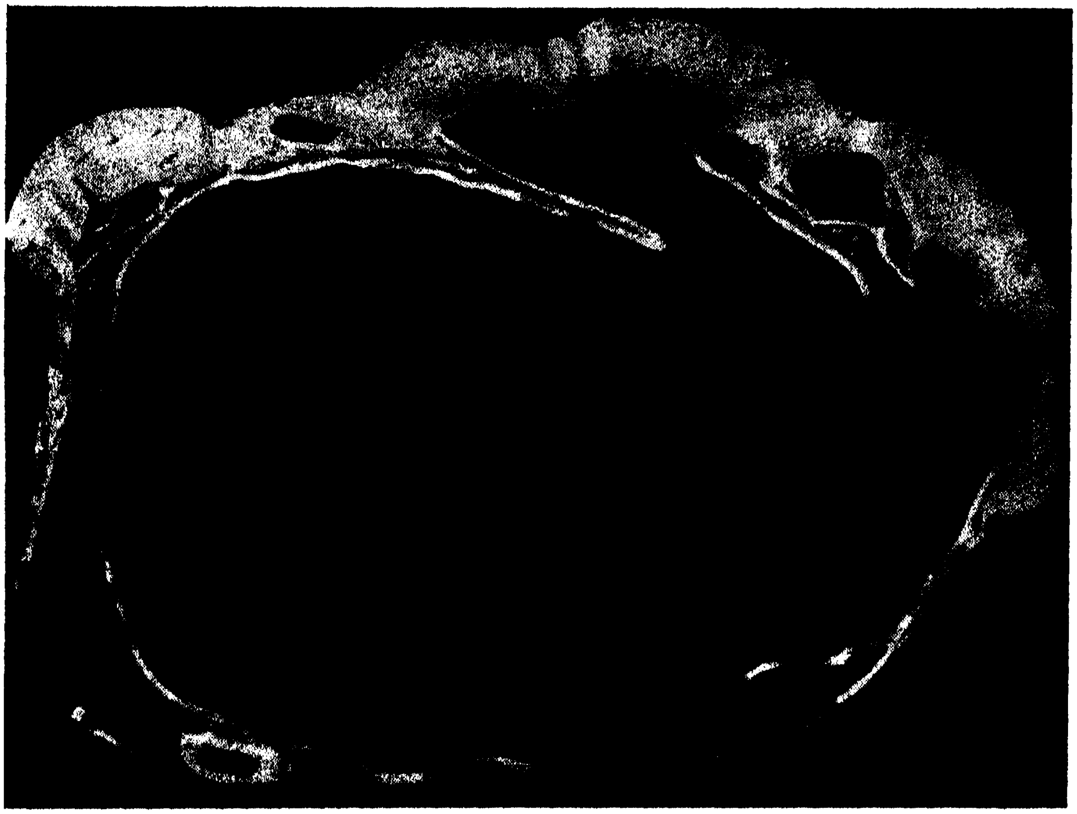
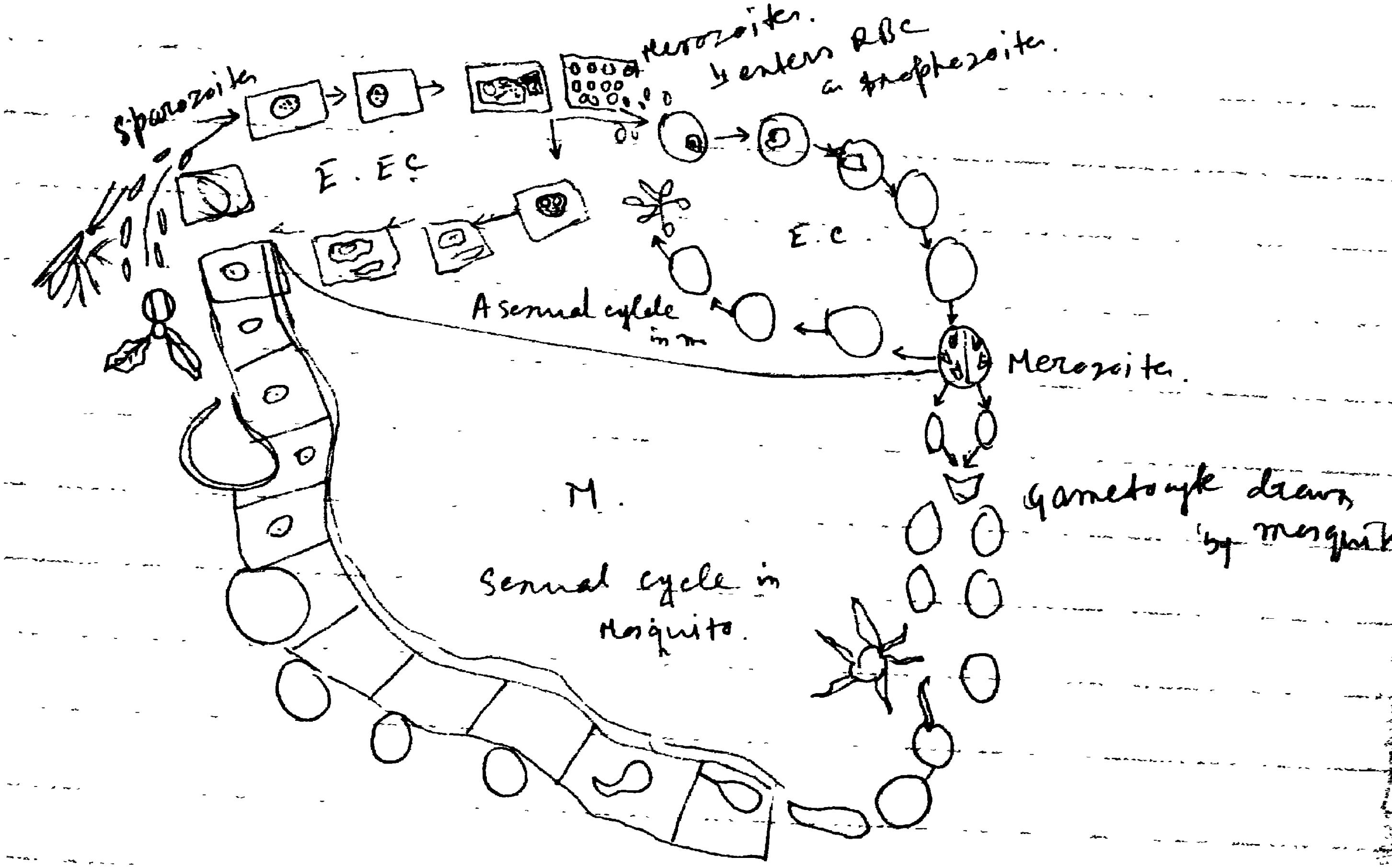


Fig. 287. Total frozen section of hydronephrotic kidney.

RETROGRESSIVE TISSUE CHANGES

Disturbance of Protein Metabolism.—Every animal cell as also the intercellular substance contains various compounds of protein, *e.g.*, albumen, globulin, nucleoprotein of the nucleus and various other protein constituents. They give us very little idea as regards their nature when we want to study their chemical or physical characters by our common histological methods, and we remain practically absolutely ignorant about the finer changes of protein metabolism by morphological studies. The only histological method to study the finer changes can be done by the process of chromolysis as described by Unna, but, unfortunately, as yet this method has been applied in the study of very few pathological conditions.


Sporozoites
Merozoites.
& enters RBC
as trophozoite.
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Asexual cycle
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Gametocyte drawn
by mosquito
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Sexual cycle in
Mosquito.

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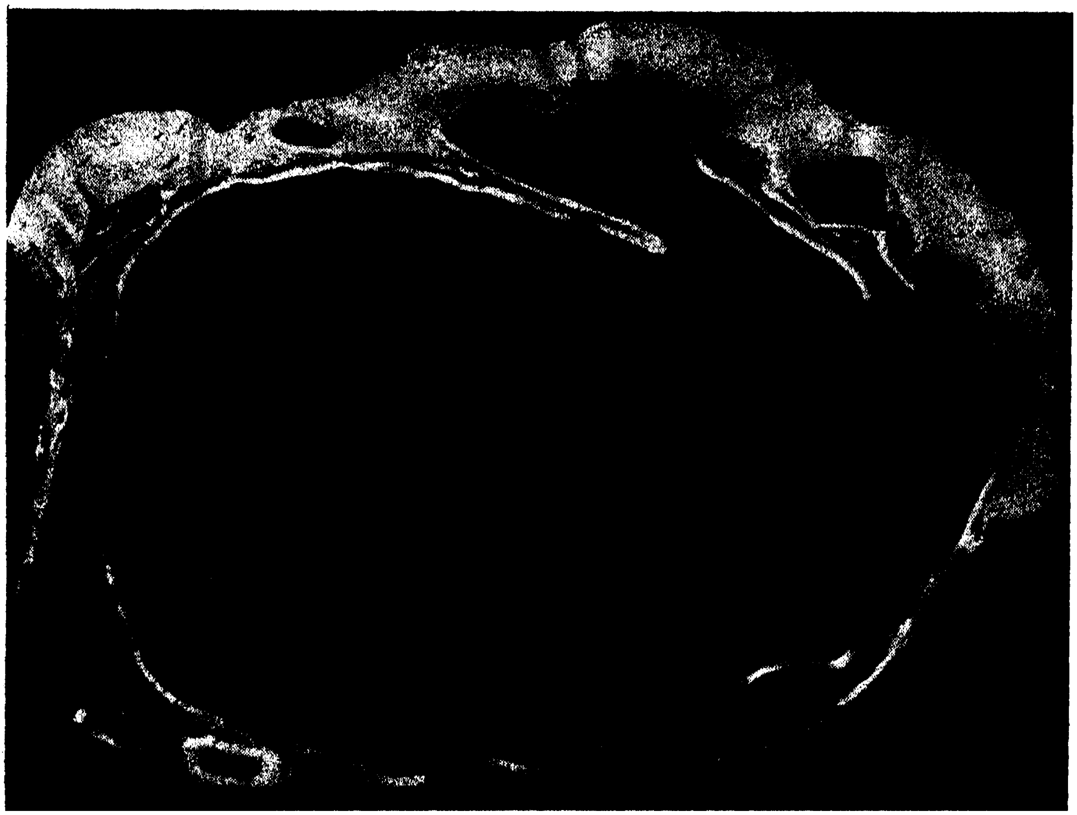

Fig. 287. Total frozen section of hydronephrotic kidney.

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CLOUDY SWELLING

(*Syn.—Parenchymatous degeneration, Granular degeneration, Albuminoid degeneration, Albuminous infiltration.*)

Cloudy swelling is an affection of the cells with the formation of cloudy appearance due to the presence of numerous granules in the protoplasm.

Etiology.—Cloudy swelling is commonly due to the intoxication of the cell by any toxic agent circulating in the blood. The toxic agent may be:—(1) Of bacterial origin, such as in pneumonia, typhoid fever, diphtheria, septicæmia, etc., (2) Derived from chemical agents, as from alcohol, phosphorous, corrosive sublimate, arsenic, snake venom, etc. (3) Produced inside the body, either from disturbed metabolism, *e.g.*, in nephritis, or in what is known as auto-intoxication. (4) High temperature which itself often causes cloudy swelling.

Mechanism of its Production.—This process was first described by Virchow who regarded it to be the mildest grade of injury to the cell. He believed that the toxic agents act on the cell protoplasm, interfering with its nutrition and so the cells are stimulated to take an increased amount of nutritional element, resulting in the enlargement of its size and increase in the number of protein granules.

Now, it is settled that this is not the increase in the amount of the protein granules, but swelling of the same. In majority of instances, it is of post mortem appearance, due to the action of proteolytic ferments. For this reason, the diagnosis of cloudy swelling is of no practical importance, unles the post mortem examination is done immediately after death and the tissue immediately fixed.

Seat of Occurrence.—The epithelial cells, mainly of the organs of elimination, *e.g.*, the kidney, liver, etc., the other structures affected are the epithelium of other glands and mucous membranes, and muscle tissue, especially of the heart.

Morbid Anatomy.—The organ is slightly enlarged, soft, inelastic and pale. On section, the cut surface shows a very opaque and dull appearance and looks as if the organ had been cooked. Occasionally there may not be any visible change to the naged eye.

Morbid Histology.—The cell is irregular, ragged, larger than normal, and, in the case of the kidney, the epithelial cells are, on account of their swelling, seen projecting far into the lumen of the tubules and occasionally meet the opposite cells, thus narrowing the lumen to a considerable extent. The affected cells are seen to be filled up with a large number of very small colourless granules which often amount to such an extent as to obscure the nucleus. This only takes place when the process is far advanced. The nuclei show no change in the beginning, but, sooner or later, they do not take the stain properly, and in very advanced condition they are not stained at all. The cells at this stage show tendency to desquamate from the lining membrane.

Although in advanced stages the nucleus is neither visible nor takes the stain, it is not absolutely destroyed. This is shown by the fact that when the granules are dissolved by dilute acetic acid the nuclei often show themselves.

Pathological Chemistry.—The granules are protein in nature. They give a positive xanthoproteic reaction, that is, when the section is put in dilute nitric acid, the granules are coloured yellow, which changes to orange red when put in ammonia. The granules are soluble in dilute acetic acid and dilute alkalies. They are insoluble in water, alcohol, chloroform and ether.

Termination.—This is the mildest form of degeneration of the cell and it recovers as soon as the acute infection or toxæmia is past. When the toxin is acting for a fairly long period, it may terminate in the actual death of the cell.

MUCOID DEGENERATION

Mucoid degeneration is the conversion of the cells and intercellular substance into a soft, semifluid, viscid, gelatinous substance, containing mucin.

Etiology.—Affection of the cells, specially the epithelial ones, of the mucous membrane is generally due to catarrhal inflammation. Mucoid degeneration is also found in cysts with epithelial acini and in the cancer cells. The intercellular material is affected in cases of myxomatous tumours, gumma, myxœdema, ovarian cyst, etc.

Morbid Anatomy.—The affected tissue looks like a homogeneous, mucilaginous, soft, jelly-like material. When localised, it appears as a cyst.

Pathologic Histology.—(*a*) *When the cells are affected.*—The epithelium of the mucous membrane shows a clear drop of mucus appearing in the protoplasm with formation of a cyst-like appearance —the goblet cell. The mucoid material increases, till the cell bursts with evacuation of the mucus. The nucleus at first seems to remain intact but subsequently disappears, leaving the whole cell to be transformed into mucus. (*b*) *When the intercellular material is affected.*—It resembles an œdematous tissue and is normally met with in the vitreous humour of the eye, Wharton's jelly of the umbilical cord and the fringes of the synovial membrane.

Chemistry.—There are three closely allied substances, namely mucin, pseudomucin and paramucin. Mucin is a form of protein (glycoprotein). It swells up with water and is precipitated by acetic acid and alcohol, but the former does not precipitate pseudomucin (as in ovarian cyst). It stains red with mucicarmin or thionin.

Varieties.—They are of two varieties:—(1) *Epithelial mucus.*—Found normally in mucous glands and increases in all catarrhal inflammation. In this case, mucin is produced no' by cell degeneration but as a result of increased secretion. (2) *Connective tissue mucus.* —Usually called myxomatous tissue. (*i*) Physiological.—The whole connective tissue during embryonic life consists of mucous tissue. In adult connective tissue also, the mucin can be detected chemically. (*ii*) Pathologically.—(*a*) Pedunculated tumour, *e.g.*, polyp. (*b*) Adenofibroma of the breast. (*c*) Myxoma. (*a*) Hydatidiform mole.—It was formerly believed to be a form of myxoma. Now it is regarded to be due to transformation of connective tissue into mucoid tissue by circulatory disturbances. (*e*) Myxœdema.—In this condition there is an increase of mucin in the connective tissue.

HYALINE DEGENERATION

Hyaline degeneration is the conversion of the cell into a transparent glass-like substance, so that it looks like an empty space to the unaccustomed eye.

Morbid Anatomy.—The hyaline substance looks like a homogeneous transparent substance, and is of globular, flat or branched trabecular form. It has a great affinity for acid aniline dyes and stains red with Van Gieson, light yellow with potassium iodide and greyish-blue with basic aniline dyes which have a violet component, *e.g.*, methylviolet, gentian-violet, kresyl-violet.

Chemistry.—It is a form of protein, insoluble in water and alcohol, and is affected with difficulty by protein-dissolving substances, *e.g.*, acids, alkalies, ferments, etc.

Seat of Occurrence.—1. INTRACELLULAR—(*i*) *Epithelial*—Produced from the cells either in the form of a secretion or as a product of destruction of the cell. It appears physiologically, *e.g.*, in colloid of thyroid and hypophysis, or, pathologically as in cysts, or, as hyline casts of the kidney. Hyaline cast was formerly believed to be produced by the coagulation of the inflammatory exudate in the renal tubules. Lubarsch thinks this to be a form of secretion of the renal epithelium and produced on account of the swelling of the cell granules, and, liberated into the tubules. This means that the renal cells are somewhat affected but has nothing to do with inflammation. (*ii*) *Of connective tissue origin.*—In this group belong the so-called Russell's Fuchsin bodies. These are round, brilliant bodies, of various sizes, and are often found in groups. They were first observed in urinary bladder. They are products of cells (some say, connective tissue, others plasma cells). They are often found in large numbers in chronic inflammatory processes and sometimes in tumours, as intracellular bodies.

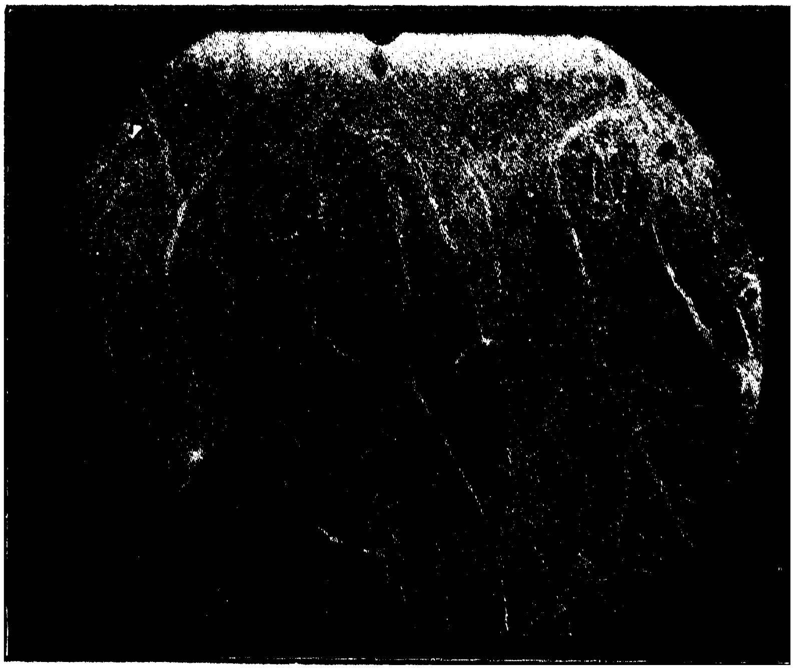


Fig. 288. Hyaline degeneration of the heart muscle

2. Extracellular—

(*i*) *Hæmatogenous.*—This has nothing to do with cells. It is formed outside the cells in the process of coagulation and produced by the *change of fibrin.* (*a*) Intravascular—*e.g.*, in hyaline thrombus. (*b*) Extravascular—*e g.*, in the form of pseudomembranous inflammation (fibrinous or crust forming) as in fibrinous pleurisy. (*ii*) *Conjunctival.*—This is formed by the transformation of connective tissue fibres. It is present in the chronic inflammatory processes (tuberculous lymph gland) glomerules of the kidney, plain muscle, small blood vessels (arteriosclerosis, psammoma, cylindroma), striated muscles (Zenker's necrosis of the rectus in typhoid fever), Weil's disease, influenza.

AMYLOID DEGENERATION

Amyloid degeneration, also known as the waxy, albuminoid, lardeceous or "bacony" disease, is the deposition of a peculiar substance called amyloid into the tissues. It is called amyloid on account of its fancied resemblance to starch. It resembles in every way to coagulation hyaline, excepting, only in the staining peculiarities.

Etiology.—Amyloid degeneration occurs in cases of long continued albuminous drainage, as in chronic suppuration, specially of the bones, chronic tuberculosis, chronic dysentery, chronic nephritis, chronic syphilis, etc. It requires at least two months from the commencement of the pathological process till the first appearance of the amyloid material in the organ. Its occurrence has been rendered extremely rare now-a days, on account of the improved surgical methods which has rendered chronic suppuration a minimum. It is produced by the excretion of circulating protein or ferment, probably of nucleoproteid in nature. For this reason, amyloidosis is so often found in suppuration, where a large number of leucocytes is in destruction. Davidson found that, after removal of the spleen in mice, no more amyloid is produced, and so it is supposed that the spleen is the source of this ferment.

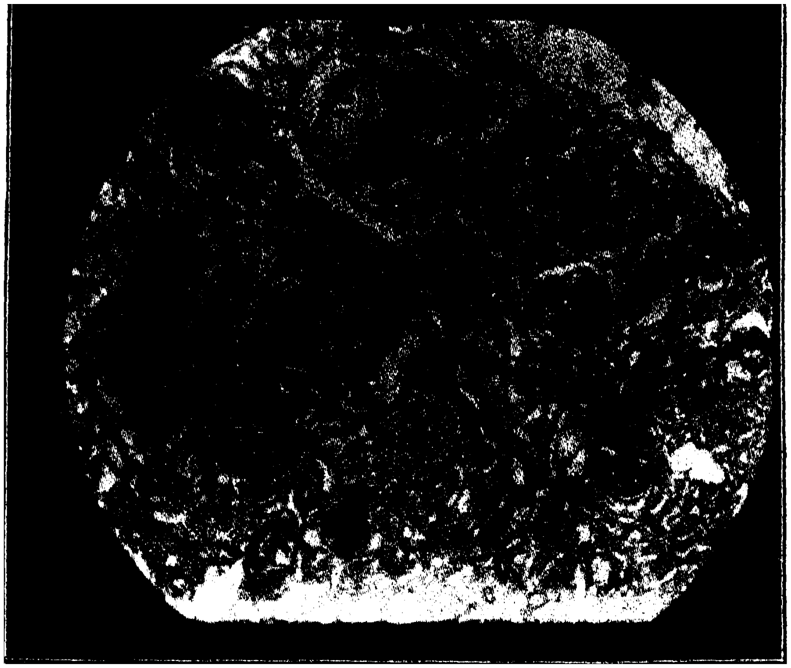


Fig. 289. Amyloid kidney.

Seat of Occurrence.—Amyloid affects the interstitial tissue and not the parenchymatous cells. The commonest organ affected is ~~the spleen~~, probably due to some ferment produced by it. Then come the suprarenal (only in the cortex), kidney, intestines, the liver, the lymphatics and other tissues. It is extremely rare in the lungs. It affects specially the media and the intima of blood vessels and the supporting framework of organs resulting in atrophy of the parenchymatous cells.

Morbid Anatomy.—In early stages there may not be any marked visible change; but in advanced condition, the organ is seen to be bigger, rigid, inelastic, with the sharp edges rounded off. The organ cuts normally and it looks like a translucent, homogeneous, glistening substance. The organ is anæmic and a smaller quantity of blood flows from the cut surface. The consistence is tougher. When diffuse, as in the case of liver, it appears homogeneous, but when it is confined to a particular structure, *e.g.*, the Malpighian bodies of the spleen, it appears as rounded translucent globules against the red background of the spleen tissue, and the characteristic appearance is usually termed as "sago spleen," on account of its appearance, resembling boiled sago grains. In the spleen, it also occurs in a diffuse form. In the kidney, the capillary tufts of the glomerules are first affected, gradually spreading to the interstitial tissue of the kidney.

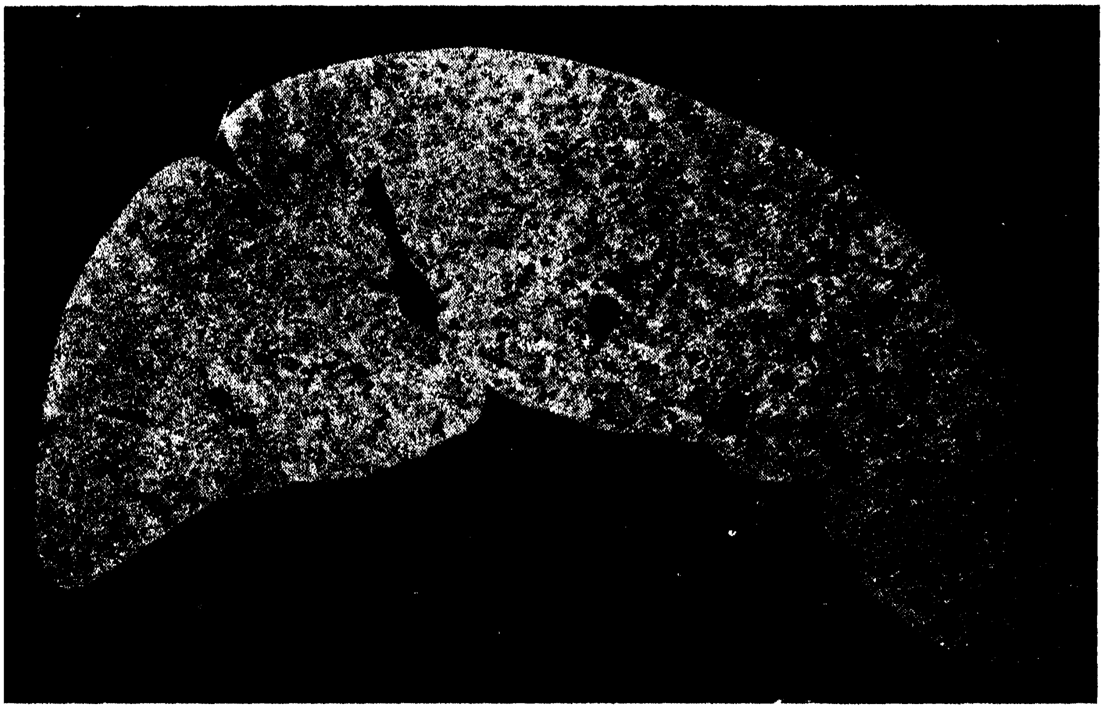


Fig. 290. Total frozen section of amyloid spleen.

Morbid Histology.—The amyloid appears in the form of layers or clumps or much branched trabecules, but never globular. As the amyloid material is mainly deposited in the small or middle-sized blood vessels and supporting framework of the organ, the walls of the blood vessels are found to resemble structureless, homogeneous cylinders, with partial narrowing of the lumen. It is present *between the cells* and never inside the cells. The same change is also found in the interstitial tissue. In the liver, it starts in the intermediate zone of the lobule, on account of the richness of the capillaries and then spreads towards the peripheral and the central parts of the lobule.

Microchemical Reactions.—Its detection becomes very easy either macroscopically or microscopically, by means of its peculiar chemical reactions. By Van Gieson stain, amyloid becomes yellow to orange-yellow.

(1) When a fresh tissue, free from blood, is dipped in a solution of iodine (iodine 1 part, ~~potassium iodine 2 parts,~~ and water 17 parts), the amyloid substance stains mahogany brown, distinctly contrasting with the yellow-stained normal tissue.

(2) When the tissue is put in iodine solution and then into dilute sulphuric acid, the amyloid substance becomes blue.

(3) Various aniline dyes, such as methyl violet and methyl green, cause the amyloid to show a metachromatic stain. The amyloid tissue is stained red, while the normal tissue becomes green, yellow, violet, etc., according to the dye used.

Pathological chemistry.—The composition of the amyloid material is still unknown. Oddi discovered that all forms of amyloid contain chondroitin sulphuric acid, a substance normally present in the cartilage. Amyloid is a compound of that acid and a protein (histone). Amyloid material is insoluble in water, dilute acids, alkalies, alcohol, chloroform, ether, xylol, benzol, etc. It resists gastric digestion, but can be slowly digested by means of artificial gastric juice.

Pathological Physiology.—*Disturbance of function.*—(1) Of the spleen and suprarenal—None; (2) Kidney—Great disturbance; (3) Liver—Extensive atrophy of the liver cells; (4) Intestine—Sometimes intense diarrhœa.

Experimental Production.—It is difficult to produce experimental amyloidosis in dogs and rabbits, but it is very easy with the hen and white mouse. This can be produced by injection of living or killed culture of saphylo-, strepto-, or gono-coccus.

Local Amyloidosis.—Local accumulation of large quantity of amyloid can occur, even in the form of tumour (rare). They may occur in the conjunctiva (trachoma), laryngeal mucous membrane, urinary bladder, etc.

Corpora Amylacea or Amyloid Bodies are laminated bodies, like starch granules, the size varying from double the size of red blood cell to a pin-point, and have no relation with amyloid disease, either in structure or in composition. Amyloid bodies are found in the central nervous system, prostate, lymphatic glands, etc., and in chronic suppuration of joints. With KI they give a changing colour varying from light-brown, deep-brown, bluish violet to black, hence are termed by some authors as *corpora versicolorata.* They stain red with Best's carmine.

Amyloid bodies are found—(1) Physiologically—In prostate, mucous membrane of the urinary bladder and pelvis of the kidney and in the central nervous system. (2) Pathologically—(*i*) In the lung—(not very often) in the neighbourhood of pneumonic or bronchopneumonic areas. (*ii*) In all cysts, especially of urinary bladder, kidney, ovary, etc.

COLLOID DEGENERATION

Colloid Degeneration consists in the conversion of the cell substance into a translucent, homogenous, structureless, gelatinous material.

Etiology.—The cause of this form of degeneration is not known.

Seat of Occurrence.—It affects the cells and occurs in the carcinoma, especially of the stomach, both in the primary and secondary growths It also occurs in the thyroid gland and in the tubules of the kidney in nephritis.

Morbid Anatomy.—The colloid material looks like jelly of various consistency and is a colourless or pale yellow, homogeneous, glistening substance.

Pathological Histology.—The cell, specially the epithelial cell, shows the appearance of one or two small droplets of colloid material. They coalesce and form bigger droplets; thereby the nucleus is pushed to one side and the cell considerably enlarged. Ultimately, the whole cell, along with the nucleus, is converted into a colloid material, and when several contiguous cells show similar changes, masses of colloid material with occasional cavity formation, occur.

Microchemistry.—The term *colloid* includes a number of different compounds and is closely related to mucoid, which led some authorities to classify them together as mucinoid degeneration. The chemical difference between the two is that the mucoid material swells in water and is precipitated by alcohol and acetic acid, whereas the colloid material does not swell in water and is not precipitated by alcohol and acetic acid.

DISTURBANCES OF FAT METABOLISM

General Remarks.—Only some portion of fat or fat-like substances of the body is visible, either with the naked eye, or by microchemical methods. On chemical examination of an organ, showing no evidence of fat under microscope, the fat-content shows 20 per cent. or more of the dried substance. This shows that the fat substances exist either (1) Dissolved in the tissue fluids, or (2) In the form of very fine invisible colloidal state, or (3) In combination with protein, rendering it unaffected by histochemical methods.

The terms "fatty infiltration" and "fatty degeneration" are not now used by the pathologists. They were originally used by Virchow, but as these conditions cannot be differentiated from each other, and more specially from experimental evidences, the two terms have now ceased to be used. (For discussion see below.)

Etiology.—Fatty changes may be physiological or pathological. In physiological fatty infiltration, there is deposition of fat derived from the circulation in the tissues which normally contain fat, as well as in those which normally contain none.

Physiological Fatty Changes.—(*a*) Subcutaneous, subserous, submucous tissues, etc.; (*b*) In liver or intestinal epithelium; (*c*) In hyaline cartilage.—This is present in all such cartilages and also during all the stages of development; (*d*) In striped muscle fibres. Here it is much irregularly distributed but almost never absent. (*e*) Testes.—From the commencement of puberty till very advanced age. (*f*) In other organs.—To a small extent regularly. (*g*) In the fœtus.—In the cells of the heart and skeletal muscles, skin, connective tissue cells and leucocytes.

This physiological deposit of fat may be further differentiated into—(*a*) Hungerfast fat, *i.e.*, the fat which is not used even by extreme grade of inanition or exhaustion, *i.e.*, in the cartilage cells, muscles; (*b*) Wandering fat, *i.e.*, the fat which acts as fat depôt and is burnt up when necessary.

When shall we Regard Fatty Changes of an Organ as Pathological?

This depends on—(*i*) The affected organ (*see* above); (*ii*) Localisation in the organ, *e.g.*, fatty changes of the peripheral zone of the liver lobule or the intestinal epithelium after a fat rich food is physiological, those of the central liver cells and Kupfer's star cells are pathological; (*iii*) Age of the individual.—Fatty liver of sucklings is physiological.

Pathological Fatty Changes.—These are due either to—(1) Overnutrition, or to (2) Malnutrition, or the action of various poisons.

(1) Overnutrition usually leads to fatty deposits in different parts of the body. This is generally due to—(*a*) Intake of excessive quantity of fatty substances as a part of natural diet; (*b*) Intake of normal quantity of fatty substances in the presence of other contributing factors such as lack of exercise, etc.; (*c*) Intake of alcohol and similar substances which in proper doses favour digestion and hence overnutrition. Alcohol itself, at the same time, aids in the formation of fats and thus predisposes to the fatty deposits; (*d*) Heredity also predisposes to fatty changes, as in obese families, the members of which are liable to this condition.

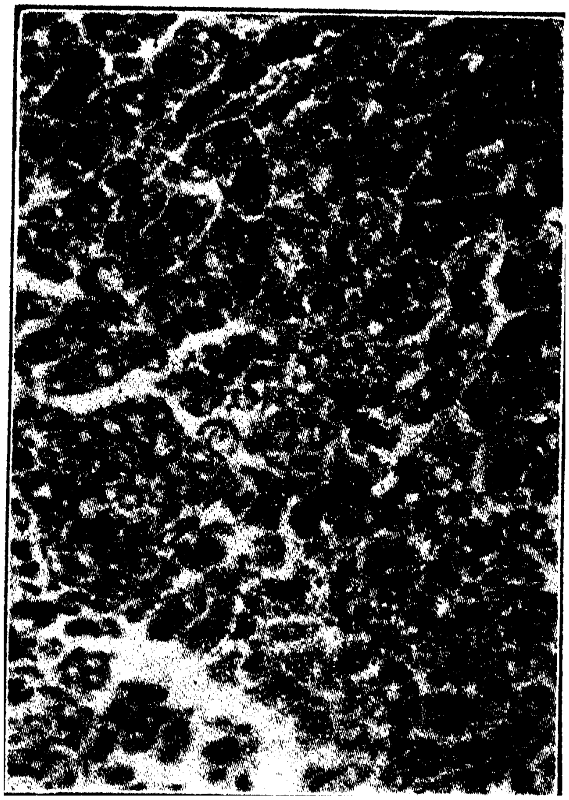


Fig. 291. Fatty liver.

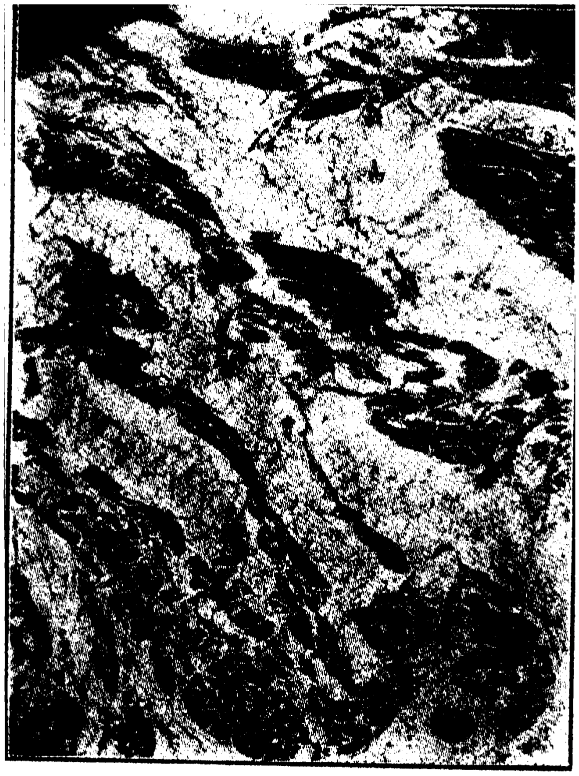


Fig. 292. Fatty heart.

(2) Pathological fatty infiltration is always due to malnutrition, and insufficient oxygenation of the tissues. Fat accumulates when the oxidative power of the cells is decreased. This takes place not by the conversion of the cell protoplasm into fat, but by deposition of fat into the cells from the circulating blood under the influence of the lipase of the cells. When there is no serious injury of the cells, fat is deposited in big droplets; but when the injury is greater and the cytoplasm undergoes autolytic changes, the fat droplets, instead of being in big drops by fusion, remain discrete small granules. (*a*) *Anæmia.*—This leads to imperfect combustion of fats in the tissues, resulting in fatty infiltration. In pernicious anæmia the distribution of the fatty changes is in places furthest from the arterioles; (*b*) *poisons.*—These are either chemical, *e.g.*, phosphorus, chloroform, antimony, etc., or bacterial, *e.g.*, in typhoid fever, diphtheria, smallpox, septicæmia, etc.; (*c*) *cachexia*, as in pulmonary tuberculosis.—There is often extensive fatty infiltration of the liver; (*d*) *gastro-intestinal affections* with exceptional digestive power with or without restricted combustion often lead to fatty infiltration of the tissues; (*e*) *excessive disintegration of fat* in the body, as in cases of fracture, diseases of bone, diseases of the nervous system with disintegration of myelin, the liberated fat is taken up by the circulation and deposited in other parts; (*f*) *accumulation of fat* takes place as a substitute or packing material in various parts of the body. This is exemplified in cases of removal of the kidney where the space is being subsequently filled up with fat. Fat also accumulates in the perinephric tissue in cases of contracted or sclerosed kidney. The space surrounding the atrophied structures, such as the muscle fibres, liver cells, etc., due to whatever cause, is often filled up by deposition of fat.

Seat of Occurrence.—The subcutaneous, subserous, submucous, intermuscular tissues, the fascia and the areolar connective tissue are commonly affected. It also affects the cells and the intercellular substance of the liver, heart and the kidneys.

Morbid Anatomy.—The fat may infiltrate the tissues, either locally, or along the bands of fibrous tissue, or in a diffuse manner. The tissue is increased in bulk and looks yellow, on account of the deposition of fat. The yellow colour may be uniform or localised, according to the diffuse or localised deposit of fat. In case of the liver, the fat is deposited along the periphery of the lobules, giving a characteristic appearance, the so-called 'fatty nutmeg liver.' The sharp edges of the organs are rounded off. During handling and cutting, the affected tissue, the fingers and the knife become greasy and show evidence of fat.

Morbid Histology.—The infiltration is mainly found in the connective tissue, rather than the parenchyma, except the liver and other organs.

In unstained preparation.—(*a*) As small drops—looks like dark black areas, but, on high magnification, becomes lighter but not brilliant. (*b*) As big drops—the dark black appearance is not so intensive. On high magnification, the middle portion is seen as transparent, the margin being formed by a dark ring.

In stained preparation.—The fat and fat-like substances (lipoid) take specific stains. They are stained red with Sudan III and scarlet red. The neutral fat can be differentiated from lipoids by staining reactions with nile-blue sulphate, by which the former is stained rose-red and the latter violet.

The fat is seen under the microscope as distinct drops, surrounded by a cell wall. The drops vary considerably in size and sooner or later, the contiguous fat cells run together, forming one big drop. The nucleus is pushed to one side against the cell wall which encloses the fat drop, giving it the appearance of a "seal ring," the seal being represented by the nucleus.

As the process advances, more and more fat droplets coalesce together to form bigger ones which are often seen faceted, on account of their mutual pressure. The largest form of fat cells is seen in lipoma.

In spite of such an extensive accumulation of fat cells in the vital organs, their functions seem to be unaltered. In very extreme cases, the fat may be very bulky so as to interfere with the nutrition of the cell and hence its function. Fatty crystals are often seen.

Morbid Physiology.—Usually, the tissue affected with the fatty infiltration does not suffer from any gross functional disturbances, except that due to the mechanical pressure. This mechanical embarrasment may, however, in extreme circumstances, lead to serious disturbances, particularly in the heart and other vital organs. Complete recovery takes place in all instances, except when the process is very extreme.

Chemistry.—The chemistry of fat is not uniform. This can be grouped as follows:–

(1) Phosphorus and nitrogen-free fat.—This is the neutral fat (triglyceride of olein, palmitin and stearic acids). This forms the main bulk of our body fat and gives the histochemical reaction of fat. To this group also belong the free fatty acids (not present in normal organism) and their Ca—, Na—, or K— soaps and the cholesterin.

(2) Phosphorus-containing but nitrogen-free fat—the so-called cerebrocides of the brain.

(3) Phosphorus and nitrogen containing fat (Phosphatids)—the most important is lecithin.

Fat is soluble in absolute alcohol, chloroform, xylol and very readily in ether. It is insoluble in water, acids and alkalies. It stains red with Sudan III and scarlet red, and stains black with osmic acid (1 per cent. solution).

Adipocere.—This is a waxy substance produced from fat, when dead bodies, especially the fat ones, are preserved in damp places. This prevents the body from undergoing putrefaction. The product consists of a mass of mixture of fatty acids and soap. This mass gradually replaces the muscles and other soft parts.

Fatty Infiltration vs. Fatty Degeneration

Virchow in the year 1847 differentiated two forms of fatty changes—(*i*) fatty infiltration, (*ii*) fatty degeneration, and this classification has since become classical.

(*i*) *In Fatty Infiltration.*—The cell is enlarged and the whole content consists of one big drop of fat which displaces the nucleus and the protoplasm of the cell to the margin, giving it the appearance of a "seal-ring," the seal being represented by the nucleus. Virchow regarded that the fat has entered into the cell from without.

Mechanism.—The cells take the dissolved fat from the tissue fluid and change it into insoluble form as a big drop. This takes place normally during digestion in the liver and intestinal epithelium. Similar changes in organ other than the liver and intestine are regarded to be pathological.

(*ii*) *In Fatty Degeneration.*—The cells are small and are filled up with numerous fat drops which remain isolated, showing no tendency to coalesce. The position of the nucleus is not atlered. Occasionally one can see finest fat droplets surrounding the cells.

Mechanism.—It is due to the change or metamorphosis of the specific protein granules of the cell into fat.

How did Virchow Establish his Teachings on Fat Metamorphosis?

(1) Fat metamorphosis is always preceded by cloudy swelling, proving the fatty changes to be necrotic in nature.

(2) The fat droplets are of same size and number as the protein granules.

(3) It was believed that the cells were able to transform protein into fat. (This was the main support for Virchow's comprehension on fat metamorphosis.)

Experimental Basis on the question of Transformation of Protein into Fat

(1) Voit and Pettenkoffer found deposition of fat in dogs fed with fat-free meat. This obseivation was for a long time accepted as correct, until it was refuted by Pfluger, who says that the supposed fat-free meat always contains a sufficient amount of fat to spoil the experiment.

(2) Milk secretion.—This was regarded as the result of fat metamorphosis and destruction of the gland cells, liberating the fat free in the secretion. Now it has been settled that the secretion of milk, as also the fatty deposit in organs after phosphorus poisoning, are not the transformation of cell-protein into fat, but are derived from the dissolved fat already present in the tissue fluids; so it is not fat-metamorphosis but fat-infiltration. This fact has been proved by numerous other experiments as follows:—

(3) Rosenfeld starved pregnant bitches, until all the fat depôts were exhausted. They were then fed with foreign fat which could be easily identified chemically. In the milk the foreign fat was found.

(4) He made similar experiments with dogs who were straved and then their own fat was replaced by foreign fat by feeding. They were then poisoned with phosphorus. In the fatty organs the foreign fat was detected and not the dog-fat which is expected to be found if the cell protoplasm has been transferred into fat.

(5) Taylor killed several frogs, dried and powdered them. By chemical examination the ratio of protein and fat was determined. A similar number of frogs were then poisoned with phosphorus and were then killed, dried and powdered. The ratio of protein and fat remained unaltered.

From these experimental facts, it is evident that Virchow's conception of fat metamorphosis, *i.e.*, the transformation of cell-protein into fat, is not valid.

The Modern Conception of Fatty Changes

The fat always comes from soluble-fat circulating in the tissue fluids.

In fatty changes of organs it depends on various factors which Lubarsch classifies as:—

(1) *Fatty Infiltration.*—This takes place when the tissue-fluids are overladen with fat, as in cases of diabetes (lipæmia), where the cells are incapable of rapidly oxidising this enormous accumulation of fat. It must be remembered that the cells are perfectly normal without any disturbance of function. *Example.*—Fatty infiltration of renal epithelium in diabetes mellitus.

(2) *Fat Retention.*—This occurs in cases where the cells are injured by some reason and so rendered incapable to burn up the fat, thus allowing them to be readily detected on histological examination. *Example.*—Phosphorus poisoning. In phosphorus poisoning two factors come in force—(*a*) fat retention, in combination with (*b*) overloading of the tissue fluids with fat from the fat-depôts (so-called *fat wandering*), rendering the organs very rich in fat.

(3) *Fat Resorption.*—In many cases, the cells attempt to carry away masses of fat deposited in some parts of the body, due to various processes, *e.g.*, resorption of fat embolus, in the margin of an infarct, in case of softening of the white matter of the brain, etc.

In these instances it is an active resorption process. The fat is taken up by the cells in the form of numerous fine fat particles (the so-called *granular synthesis*).

(4) *Fat Secretion.*—This is exemplified in case of milk secretion which is purely a secretory process with no destruction of glandular epithelium.

(5) *Fat Phanarosis.*—This means that the unseen fat elements are rendered visible, probably by some autolytic process. This does not play any important part in human pathology.

LIPOID INFILTRATION

Lipoids are substances which resemble fat closely, but differ as follows:—(*a*) They are doubly refractile (anisotropic) and can be easily recognised by the polarising microscope; (*b*) they are capable of forming transparent rhombic flat crystals often with broken edges;

(*c*) chemically they are mixed up with cholesterinester; (*d*) by staining reactions, they behave generally like fat, except with nile-blue which stains fat—red and lipoids-violet.

Seat of Occurrence.—(1) *Physiological.*—Suprarenal cortex, corpus luteum, puerperal uterus during involution, mucous membrane of the gall bladder, interstitial cells of the testes.

(2) *Pathological.*—(*a*) Chronic inflammation of female genital organs; (*b*) atheroma of the arteries—atherosclerosis; (*c*) kidney—so-called large white kidney; (*d*) hæmorrhagic pachymeningitis; (*e*) diabetic xanthoma.—These are yellow tumour-like masses on the skin, due to the stasis of the lipoid-containing lymph, resulting in proliferation of lymph endothelium in the form of new growth. In diabetes the blood and lymph are abnormally rich in lipoids (lipoidæmia).

Lipins.—These are substances which include neutral fat, fatty acids, soaps, lipoids and their compounds.

Lipæmia.—Normal blood contains a considerable amount of fat, and it increases considerably after a fat-rich meal. Lipæmia generally occurs in diabetes.

DISTURBANCES OF THE CARBOHYDRATE METABOLISM

Glycogen Infiltration

Etiology.—Glycogen occurs in the organs—(1) During active work, *e.g.*, in muscles, bundle of His, etc.; (2) active growth, *e.g.*, in embryo or in tumours; (3) in active regeneration, *e.g.*, postmenstrual phase of endometrium.

Morphology.—The glycogen appears as transparent glass-like drops and leads to swelling of the intercellular substance. It is found in the protoplasm of the cell and is rarely extra cellular and never in the nucleus, except in pathological conditions.

Chemistry.—It resembles starch. It is insoluble in alcohol but soluble in water, but not always so As for example, glycogen infiltration of the epithelium of the skin is not easily washable in water, unless, the piece of the skin is kept in saliva for some time. It stains specifically by Best's carmine, when it becomes red. It becomes dark brown with potassium iodide.

Seat of Occurrence.—(*a*) *Physiological.*—(1) In embryo.—In all organs, except the liver; (2) in adult.—Always in the liver, muscles, esp. Bundle of His, cartilage cells, squamous epithelium, uterus during postmenstrual phase but not during menstruation.

(*b*) *Pathological.*—(1) Metabolic disorders, *e.g.*, kidney-epithelium in diabetes; (2) certain circulatory disturbances, *e.g.*, the nucleus of the liver cells in chronic passive congestion; (3) inflammation.—In leucocytes; (4) tumours.—Hypernephroma or lieomyoma of the kidney, tumour of the suprarenal cortex.

DISTURBANCES OF MINERAL METABOLISM

Calcification

Calcification or calcareous infiltration or petrification consists in the infiltration of insoluble calcium and magnesium salts in and between the tissue cells.

Ossification consists in the deposition of calcium salts with formation of true osseous tissue, through the activity of the osteoblasts. Occasionally a calcified tissue may be converted into an osseous one.

Mineral salts are absorbed into the system through digestion of the foodstuffs containing them. These particles are not visible under the microscope normally, as they circulate, either in solution or in colloidal state, except in case of the bones. Calcium salts are normally excreted mostly through the intestines and the kidneys.

Etiology.—PHYSIOLOGICAL.—Calcification of laryngeal and costal cartilages, Hassal's corpuscles in the thymus, pineal body, ossification of the first rib, thyroid in old persons.

PATHOLOGICAL.—(1) *In necrotic and dead areas*—(*a*) dead parasites—echinococcus, filaria, trichina, etc.; (*b*) old tuberculous and gummatous lesions, old infarcts, old thrombus (phlebolith); (*c*) dead fœtus escaped from ruptured tubal pregnancy (lithopedion); (*d*) atherosclerosis of vessels; (*e*) tumours, *e.g.*, myoma of the uterus. Even cancer of the ovary and breast. Psammoma is the frequent seat.

(2) *Excretory product of the cell*—*e.g.*, exudates, transudates, secretion, etc. This commonly occurs in the form of calcareous nodules on the renal cortex (deposition of calcium salts in the glomerular space). This is due to the fact that one-third of the calcium salts is excreted through the kidneys and the rest through the intestines. For this reason calcareous infiltration is common in the kidney.

(3) *In the cells.*—This is found generally in the renal epithelium after Hg. poisoning (earliest 7 days after poisoning). The renal epithelium is also affected in cases of anæmic infarctions and chronic nephritis, with extensive necrosis of the epithelium.

(4) *In connective tissue fibres.*—The kidney also serves as an example in this case too, as in the so-called calcareous infarcts. These are white stripes which radiate from the apex of the pyramids. This shows that the calcareous particles are deposited in the fibrous tissue between the collecting tubules. This form of calcification also takes place in the pleura, dura mater, intima and media of arteries and the choroids.

(5) *In general metabolic disorders.*—*e.g.*, in myositis ossificans.

Conditions for the Deposition of Calcareous Particles.—(1) Increased affinity of the tissues for calcareous infiltration—(*a*) Physiological—bone tissue has the greatest affinity for calcareous salts; (*b*) Dead or degenerated tissue.

(2) Absolute or relative increase of calcium content of the tissue fluids. (*a*) Deficiency of excretion of calcareous salts, due to disease of the kidneys or intestines or due to increased intake; (*b*) Pathological dissolution of the bones. This process has been called by Virchow as *Kalkmetastasis.* In this condition calcareous infiltration takes place in the lungs, kidneys, heart, stomach, etc., in cases of dissolution of bones rickets, osteomalacia, ostitis fibrosa, bone tumours, etc.

Mechanism of Calcification.—There are two main views—those of Klotz and Wells. Klotz believes that calcification is always preceded by fatty degeneration of the tissues, in which the fatty acids and the neutral fat present combine with the sodium or potassium. with production of soda or potash soaps. The sodium or potassium radicles are then gradually replaced by calcium, with production of insoluble calcium soap. Ultimately, the carbonic and phosphoric acids of the blood produce the highly insoluble calcium phosphates and carbonates, by driving out the fatty acid radicles of the calcium soaps.

Recently Wells observes a different view from that of Klotz and shows that it is a process similar to that of absorption. His observations are mainly on the experimental production of calcification in different tissues implanted into the abdominal cavity of rabbits. The tissues are taken out at varying intervals and their calcium content carefully estimated. He found that the calcification is preceded by hyaline changes of the tissue which have specific affinity for calcium. The calcium salts remain in the soluble form by the carbonic acid. The hyaline substance takes up the soluble calcium, with subsequent precipitation, due to the reduction of the carbon dioxide contents. The presence of insoluble calcium salts will lead to further deposition of insoluble salts directly from the soluble ones brought to the tissues by the blood stream.

Morbid Anatomy.—The calcified tissue is hard and may be either friable, gritty or stony. This generally happens when the deposition of calcareous particles is fairly advanced and is better felt than seen. When accumulated in considerable masses, as in fibroid of uterus, it may be difficult to cut with a knife or even with a saw, due to the hardness and gritty conditions.

The delicate structures, such as the cardiac valves, arteries, etc., become very rigid, brittle, inelastic and opaque. In case of peripheral arteries it feels like a string of beads, the so-called " pipe-stem arteries " (Moenckeberg type of arteriosclerosis). In case of bigger arteries, as the aorta, the calcareous deposits, during the atheromatous condition, are in the form of scales.

Histology.—The calcareous particles may be found either inside or outside the cell—commonly the latter. In the early stages, they appear as very small irregularly scattered dust-like particles. When seen in the unstained condition, they look like small refractile granules or masses, irregular in outline, black with transmitted light and white with reflected light.

Hæmatoxylin stains the particles an intense blue-black colour. The structure of the tissue can be more clearly studied by dissolving out the calcareous particles by dilute mineral acid. In the advanced condition they appear in masses.

Chemistry.—The calcareous particles are soluble in dilute acids, with evolution of carbon dioxide, when composed of carbonates of calcium and magnesium. They are insoluble in water, alcohol, chloroform, ether, xylol, etc.

Ossification.—This depends on the deposition of bone in the tissue, with presence of lacunæ, canaliculi and Haversian systems. They are present in the following conditions:—(1) *Myositis ossificans.*—This occurs in muscles, especially of those parts which are subject to chronic irritation; (2) *Osteophytes.*—These occur in normal structures, and may not form any tumour-like mass. The falx cerebri and other parts of the meninges are affected, with formation of translucent thin bony plates; (3) *Ossification of a calcified tissue* may also take place. These are found in ossifying fibroids of the uterus, ossifying scarcoma, ossifying arteritis, etc.

Concretions.—These depend on the deposition of mineral matter in the cavities or their passages, and are usually preceded by catarrh of the mucous membrane of the respective situations. They occur in the pelvis and substance of the kidney, uterus, urinary bladder, gall-bladder, bile passages, pancreatic duct, salivary duct (sialolith), intestines (enterolith), in the blood vessels (arteriolith, phlebolith), etc.

Etiology.—(1) There must be plenty of dissolved mineral substances in the affected fluid; (2) The substances must be easily precipitated; (3) There must be something to form the centre of crystalisation, *e.g.*, cells, bacteria, red blood cells, exudated foreign body, hair, etc.

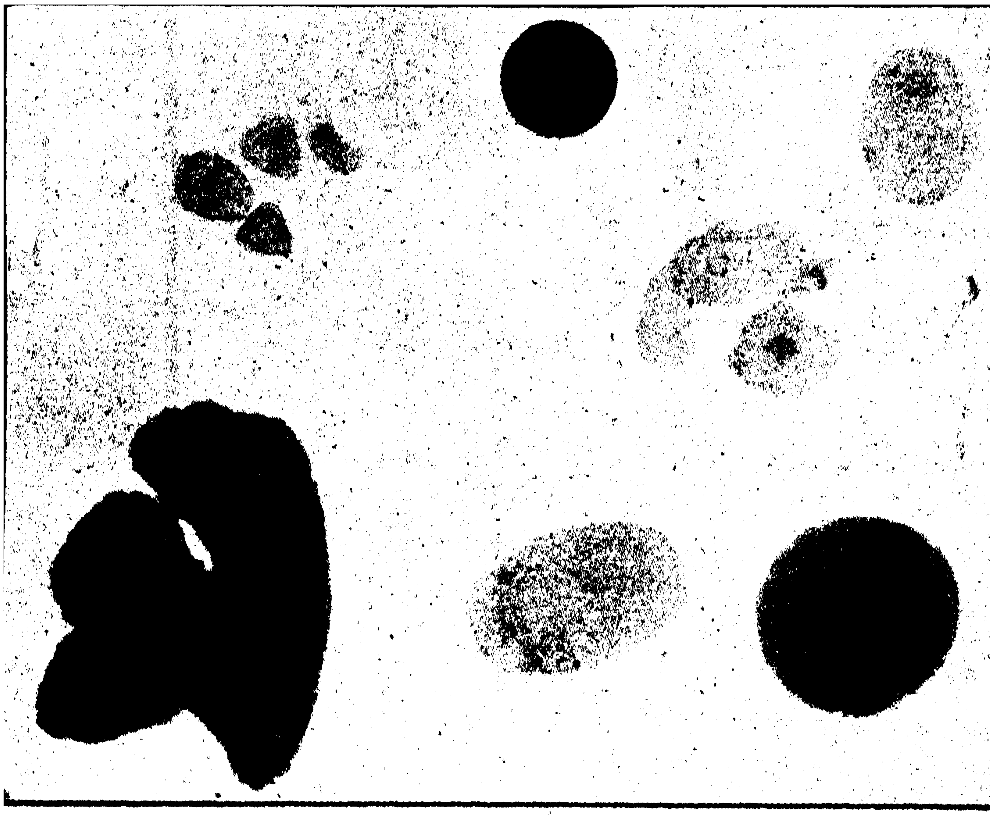


Fig. 293. A skiagram of renal, vesical, and gall-stones and a silver two-anna piece for comparison of the density of the shadows: (*a*) Silver two-anna piece; (*b*) Renal stone (from the pelvis of the kidney); (*c*) Stone from the urinary bladder; Rest—gall stones.

Gall-stone.—This is either of the following:—

(1) *Cholesterin-pigment-calcium stone.*—The commonest form of gall-stone. This is always due to microorganism. The mechanism is as follows:—First of all due to some stasis of bile, cholesterin or cholesterin-pigment stone is formed, which causes an obstruction to the

bile passages; the bile, in stasis, is easily infected, resulting in the precipitation of calcium salts. This can be experimentally proved by keeping sterile bile with certain bacteria in a test tube, when cholesterin and calcium salts will be precipitated. In contradistinction to the cholesterin or cholesterin-pigment stones, cholesterin-pigment-calcium stones are always multiple.

(2) *Cholesterin stone* (in combination with fatty acids, as ester). —This is rare and is always single, white, soft, glistening and laminated.

(3) *Cholesterin-pigment stone.*—This is very rare. This was formerly believed by Klebs to be *inflammatory* in origin, due to the frequency of the presence of bacteria. Backmeister and Aschoff are of opinion that cholesterin or cholesterin-pigment stones are always *non-inflammatory* in origin, due to the stasis of bile, leading to the crystallization of cholesterin, which has been experimentally proved by keeping sterile bile, in test tube, still for some time, when crystallization of cholesterin takes place.

(4) *Pigment stone.*—This is rare.

(5) *Calcium stone.*—Also rare.

Stones of the Urinary Tract

General Peculiarities.—(1) Each has a nucleus and a coating; (2) They are either inflammatory or non-inflammatory in origin.

General Conditions.—Different urinary salts are deposited according to the reaction of urine—(1) In acid urine—Uric acid, urates, cystin; (2) In neutral urine.—Oxalic acid, ammonium urates; (3) In alkaline urine.—Calcium phosphate, ammonium-magnesium-phosphate, xanthin.

Different Forms of Urinary Stones.—(1) *Uric acid stones.*—Composed of uric acid or urates. These are not very big, but round, reddish-brown in colour, of moderate consistency and mostly primary (uric acid diathesis); (2) *Oxalate stones.*—These are angular, rough, mulberry-form, extremely hard, and so often cause injury to the urinary passages with hæmorrhage. Due to the addition of blood pigments, they often take reddish to blackish colour. They are mostly primary stones (oxalic acid diathesis); (3) *Phosphate stones.*—These are extremely irregular, chalk-like, not very hard and mostly inflammatory in origin; (4) *Cystin stones.*—These are rare, soft and laminated; (5) *Xanthin stones.*—Extremely rare, cinnabar-red in colour.

Results of Stone.—(1) Injury; (2) Inflammation; (3) New growth formation.—In 88 per cent. of all cases of cancer of gall-bladder, gallstone is found during post mortem examination, or they show symptoms of gallstone during life.

Gouty Degeneration.—This depends on the deposition of sodium biurate in the tissues.

Mechanism of its occurrence.—Urates are the acid salts of uric acid. Uric acid is derived from the nucleoproteins of blood or of the body tissue. It is oxidised by enzymes in the liver and other organs, with the production of urea which is excreted with urine, along with a small amount of uric acid itself which has escaped oxidation. The solubility of uric acid varies in different conditions. It is held in solution in the blood, but is less soluble in urine and is almost insoluble in water.

In urine the deposition depends on concentration, acidity and other factors, and its elimination depends mainly on the power of the kidneys to excrete them.

The amount of uric acid in the blood and in the urine differs to a considerable extent and depends on factors summarised by Wells as follows:—(1) The amount of purin bodies taken in the food, upon which chiefly depends the amount of exogenous uric acid; (2) The amount of destruction of tissue nucleo-proteins; (3) The amount of purin bases formed in the muscle tissue; (4) The amount of conversion of purin bases into uric acid; (5) The amount of destruction of uric acid, if any, occurring in the body; (6) Possibly upon the capacity of the tissues to synthetise uric acid, and in case such power to synthetise uric acid exists, upon the presence of the precursors of uric acid in the body; (7) The retention of uric acid in the blood and the tissues; (8) The power of the kidneys to excrete uric acid.

In gout, deposition of uric acid, mainly in the form of sodium urates, takes place and occurs chiefly in the articular cartilages, with formation of small, rounded masses in the joint and adjacent subcutaneous tissue and the skin, forming the so-called " tophi " or chalk stones which may ulcerate subsequently. (See fig. 309-A. Page 574.)

Extraneous Mineralisation.—This depends on the accumulation of extraneous mineral matter taken and retained in the tissues. This generally takes place through inhalation of such substances and is known as *Pneumonokoniosis.* These are anthracosis, kaolinosis, siderosis, etc.

DISTURBANCE OF PIGMENT METABOLISM

Pigmentary Infiltration

Pigmentary infiltration is the accumulation of colouring matter in a tissue, as a result of altered pigment metabolism or introduced from without. Thus the infiltration of pigment in the tissues takes place from either of the two following sources—(1) Endogenous pigment, (2) Exogenous pigment.

Endogenous Pigment.—There are several kinds of endogenous pigment formation. Some tissues are normally provided with the endogenous pigment, *e.g.*, the pigmented cells of the retina and choroid of the eye, and of the skin and the hair. In some lower animals, as the chameleons, the pigment is present in the connective tissue cells and changes in colour under the action of the sun's rays. Hæmoglobin is the normal colouring matter of the blood and is also responsible for the production of bile pigment. In pathological pigmentary infiltration, the hæmoglobin is converted into the various forms of pigments.

Endogenous
  (1) Hæmoglobinogenous — hæmosiderin, hæmatoidin, malaria-melanin.
  (2) Nonhæmoglobinogenous (autogenous, metabolic) *e.g.*, atrophy pigment, melanin pigment.

(1) *The Hæmoglobinogenous pigment.*—There are several forms of such pigments, all of which are derived from hæmatin which, when combined with a globin, produces hæmoglobin. So long the hæmoglobin remains enclosed inside the red corpuscles, there is no deposition of this pigment, but as soon as the corpuscles are broken (laking of blood), the hæmoglobin comes free in the plasma and serves no physiological function and hence is eliminated from the body by the kidneys and is partly utilised by the liver in the production of bile. Its presence in the urine produces what is known as *hæmoglobinuria* and the excessive production of bile produces jaundice.

The conditions in which laking of blood takes place with liberation of free hæmoglobin are as follows:—(*a*) Introduction of distilled water into the circulation; (*b*) Organic poisons—chemicals, snake venom, etc.; (*c*) Inorganic poisons; (*d*) Introduction of foreign blood, hæmolysins or hæmolytic toxins.

The etiological factor of blackwater fever is not definitely known, but this serves the best example in which intravascular hæmolysis takes place.

The hæmatogenous pigments are the following:—

1. **Hæmoglobin.**—It is an iron-containing, red, amorphous substance, normally present in the red blood corpuscles. It is soluble in water and alkaline solution, but insoluble in alcohol, chloroform and ether.

2. **Hæmosiderin.**—It is an iron-containing, yellowish or brownish amorphous substance and is commonly found in all extravasations of blood into the tissue and hence very commonly found in ecchymosis, purpura, wounds, scurvy, hæmorrhage in the tissue, specially the brain, meninges, hæmorrhagic infarction of the lung, etc.

Apart from the local process, hæmosiderin is also found in the tissues, as a result of some general systemic condition. The most common instance is pernicious anæmia. In pernicious anæmia there is a wide-spread destruction of red blood corpuscles, liberating a considerable amount of hæmoglobin in the circulation. This leads to either excessive production of bile pigments or deposition of a large amount of hæmosiderin in the liver cells. The cells affected are generally of the peripheral zone of the lobule. The cortex of the kidney, spleen and other organs often shows the presence of hæmosiderin.

In hæmochromatosis, there is widespread deposition of hæmosiderin and another iron-free pigment, hæmofuscin, both in the liver and other parts of the body, with cirrhosis of liver and glycosuria, the so-called " bronzed diabetes."

Chemistry.—Hæmosiderin is soluble in acids, insouble in alkalies and takes the following special stains:—(1) *Perls' reaction.*—Hæmosiderin is stained blue with potassium ferrocyanide and HCl. (the so-called Prussian blue reaction); (2) *Quincke's reaction.*—Hæmosiderin stains black with ammonium sulphate; (3) *Turnblue reaction.*—This is the most delicate test for hæmosiderin which is stained blue with ammonium sulphate and ferricyanide of potassium.

Hæmosiderin is specially produced in those places, where living cells come in close association with the destroyed blood. These pigments are generally taken in by leucocytes, macrophages or retiulo-endothelial cells. These hæmosiderin-containing cells are called " sideroferic " cells.

Localisation of Hæmosiderin.—(1) Immediate neighbourhood or in the place where hæmorrhage has taken place. At least 36 hours are necessary for the hæmosiderin to appear; (2) reticulo-endothelial cells of the spleen, liver, bone marrow and lymph glands; (3) in round cells, either hæmatogenous or histiogenous; (4) in epithelium of glandular organs, *e.g.,* liver, kidney, pancreas, etc.; (5) striated muscles, also in heart muscles, but never in plain muscles; (6) in ganglion cells—extremely rare.

Mechanism.—It is differentiated into—

(1) *Hæmosiderin-resorption.*—In this case, hæmosiderin is actively taken up by wandering cells, *e.g.,* in the neighgourhood of hæmorrhagic areas.

(2) *Hæmosiderin infiltration* (Hæmosiderin metastasis).—Hæmosiderin can be found in the tissue fluids (after repeated destruction of red blood cells), and then deposited in the form of granules in the different organs, *e.g.,* in pernicious anæmia, there is hæmosiderin infiltration in the liver, kidney, heart, etc., without having any hæmorrhage taken place in these structures.

(3) In chronic congestion or hæmorrhage of the lung, hæmosiderin is taken up by wandering cells and is deposited in the alveolus and is occasionally found in the sputum. These pigment-laden cells are known as " heart-failure " cells.

In cases of excessive intravascular hæmorrhages, *e.g.,* in some infections (specially with hæmolytic streptococcus) and poisons, the hæmoglobin is broken up and produces an excess of bilirubin and shows a considerable amount of urobilin in urine. If the amount of free hæmoglobin exceeds a certain amunt, it is excreted by the kidneys, as such, resulting in hæmoglobinuria.

Hæmochromatosis.—This takes place in—(1) Glandular organs.—Liver, pancreas, kidney, etc.; (2) in lymphatic structures.—Spleen, lymph gland, bone-marrow; (3) plain muscles of blood vessels and intestine; (4) skin; (5) heart and striped muscles.

Nature of Pigment in Hæmochromatosis.—Von Recklinghausen found two forms of pigment—(1) one giving iron reaction (hæmosiderin); (2) one giving no iron reaction. Von Recklinghausen believed this to be a metabolic pigment. Schmidt regarded this to be the old form of hæmosiderin, giving no iron reaction. Lubarsch believes this undubtedly of hæmoglobinogenous origin, only, the pigment of plain muscles being of brown metabolic pigment.

Etiology.—Von Rechlinghausen took as etiological factors.—Cirrhosis of liver, diabetes, pernicious anæmia, leukæmia, tuberculosis, etc. Hanot thinks this to be due to metabolic disturbances (bronzed diabetes). Lubarsch is of opinion that this is due to—(*a*) Severe disturbance of protein metabolism; (*b*) Abnormal destruction of red blood cells due to—(*i*) Intravascular hæmolysis, (*ii*) Resorption of hæmorrhagic exudate or transudate.

3. **Hæmatoidin** (identical with bilirubin, at least an isomer of it).—It gives a rainbow colour, like bilirubin, with concentrated nitric or sulphuric acids. Unlike hæmosiderin, hæmatoidin occurs without the activity of any cell and is iron free. It is a reddish-brown, rhombic crystaline pigment and is generally found in hæmorrhages. It is soluble in chloroform but insoluble in water, acids, alkalies, alcohol and ether. It is commonly found in the chronic passive congestion of the liver (nutmeg liver).

Fig. 294. Malarial pigment in the liver.

4. **Malaria Pigment.**—This is a black pigment and is derived from the metabolic activity of the malarial parasites, while still enclosed inside the red blood cells. After the bursting of the red cell, the pigment, called the *hæmozoin,* is liberated into the circulation and is taken up by the leucocytes and other wandering cells and ultimately carried to all the organs of the body especially the spleen, liver and the brain. Malaria pigment is in close relation to hæmatoidin. It is insoluble in watery acid solution but soluble in alcoholic acid solutions. It is also insoluble in alcohol, ether, chloroform. It does not give the ordinary iron reaction, but it gives a positive reaction when alcoholic HCl is used in the Prussian blue reaction.

Hæmatin.—This is found in old hæmorrhage, but it is not a permanent pigment, being broken down into the iron-containing hæmosiderin or the iron-free hæmatoidin.

Bile Pigments.—These are iron-free derivative of hæmoglobin, produced by the liver cells, which flow through the bile passages, ultimately into the intestines. They are as follows:—(1) *Bilirubin.*—This is golden-yellow in colour and, being soluble, stains the tissues; (2) *Biliverdin.*—This is produced from bilirubin by absorption of oxygen and is green in colour; (3) These bile pigments are reduced in the intestines to hydrobilirubin or *Stercobilin,* the pigment of the fæces. The bile pigment is reabsorbed from the intestine through the portal circulation to be reeliminated by the liver, or to produce (4) *Urobilin.*

Occurrence.—(1) *Diffuse.*—Physiologically in the liver cells and pathologically in all the organs, in cases of jaundice, in the tissues as well as in the tissue fluids. (2) *In granular form.*—(*a*) In the liver cells, in cases of stasis of bile, generally combined with bile thrombus (Epinger), *i.e.*, thick bile filling up the widely dilated bile capillaries; (*b*) In Kupffer's stellate cells; (*c*) In the kidney epithelium, in the form of the so-called bilirubin infarct which occurs very commonly in the newborn along with uric acid infarct; (*d*) Nucleus of the ganglion cells of the pons and medulla of the newborn.

Causes of Jaundice.—Bile stasis, due to obstruction of the bileflow, *e.g.*, catarrhal jaundice, in gall stones, overproduction of bile; (2) Acute infectious diseases, *e.g.*, Weil's disease, septicæmia, acute yellow atrophy, pneumonia, etc.; (3) Chronic diseases, *e.g.*, heart disease, with congestion of the liver, cirrhosis of the liver, etc. (4) Poisoning—phosphorus, mushroom, etc.

Morbid Histology.—Most commonly the bile pigment occurs as diffuse staining, so that the microscopical examination of the organ does not show the presence of the pigment. In very severe jaundice, the pigment appears inside the cells in the form of fine granules.

Pathogenesis.—(1) *Hepatogenous.*—Bile is produced in the liver cells, but, instead of flowing out into the bile ducts, flows into the blood or lymph channels. This occurs in cases of—(*a*) Obstruction of the bile passages; (*b*) In cases of excessive production of bile; (*c*) Inflammatory processes, *e.g.*, Weil's disease; or, (*d*) Change of the structure of the liver, *e.g.*, cirrhosis of the liver.

McNee has produced jaundice, after removal of the liver in geese, with arsenic poisoning. This questions the teaching that bile is always produced by the liver. It has also been found that the reticulo-endothelial system of organs other than the liver, especially that of the spleen, can take part in the formation of bile. On the other hand, Whipple and Hooper experimentally proved that if the liver and all the abdominal viscera be excluded from the circulation, the blood plasma still shows the presence of the bile pigment.

Two sites are attributed to be the seat for the formation of the bile pigment:—(*a*) The liver cells responsible for the formation and excretion of the bile pigment, and (*b*) The reticulo-endothelial system of the liver, especially Kupffer's stellate cells, and also of the spleen, is responsible for the formation of bile pigment from the hæmoglobin.

Aschoff summarizes all that is known about the formation of bile pigment, specially on experimental basis, as follows:—

" In agreement with Whipple and Hooper, an anhepatic formation of bile pigment can be demonstrated. In animals deprived of their livers by the method of Mann and Magath, there occurred regularly, and within a definite time after intravenous injection of hæmoglobin, a formation of bile pigment in the blood plasma. Also, without injection of hæmoglobin, bile pigment is formed, if the animal survives the operation long enough. This formation of bile pigment in the blood could not be attributed to a resorption from the intestine, for it occurred after hæmoglobin injection in the same time-interval in animals in which the hepatic circulation was intact, as in such, in which all resorption from the intestine was excluded by tying off the portal vein and opening the thoracic duct. This bile pigment, formed anhepatically in the blood, occurs in such large quantities that the natural daily quantity of bile pigment excreted with the bile would be assured, if the animal lived long enough.

" Morphological investigations, which show the first changes in toxic icterus to involve the reticulo-endothelial elements, while the liver cells are still entirely unaltered, confirm the opinion, that the reticulo-endothelial apparatus, *i.e.*, the splenic tissue, without and within the liver, and not the liver cells, plays an important rôle in the formation of bile pigment. Only in the further course of the toxic formation of bile pigment do changes in the liver cells occur, appearing in the form of bile cylinders. They are to be looked upon as pure stasis cylinders, resulting from the tenacity of the bile. The change in the delayed bilirubin reaction of van den Bergh into the prompt reaction, seems to be connected with their appearance."

(2) *Hæmato-hepatogenous.*—This is caused by increased destruction of red blood cells, liberating more hæmoglobin for the liver to

work with, and hence overproduction of bile, *e.g.*, in newborn and in hæmolytic jaundice.

(3) *Hæmatogenous.*—In this case, the jaundice has nothing to do with the liver. It is produced in the tissues and liberated from these into the tissue fluids. This is of very little practical importance.

Icterous Neonatorum.—This is regarded to be physiological. This appears on the second or third day after birth and generally disappears after a few days. In severe cases, death may result with bilirubin infarct of the kidney and staining of the nucleus.

Icterus neonatorum is caused by the destruction of a large number of red blood corpuscles.

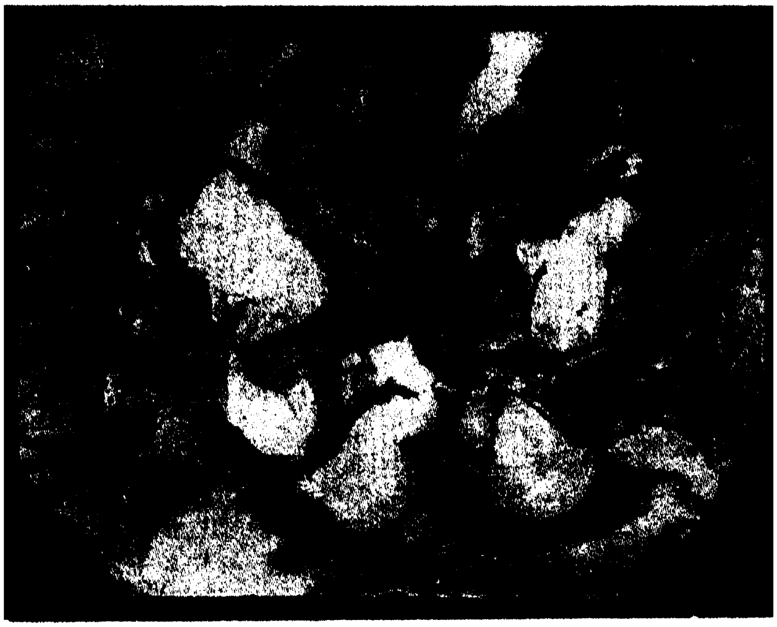


Fig. 295. Carbon particles in the lung.

Exogenous Pigments.—These are pigments derived from foreign substances introduced into the body. The more common forms are the following :—(1) *Pneumonokoniosis*.—(*a*) Anthracosis is the term used to denote the accumulation of coal particles in the lungs and other tissues, the result of inhalation of coal dust or soot, and is common among miners and town-dwellers; (*b*) Kaolinosis is produced by inhalation of clay and is common in potters; (*c*) Siderosis is produced by inhalation of the metallic dust of iron and is common in those who work with iron fillings; (*d*) Calicosis is due to the inhalation of dust from cut stones.

Mechanism of pneumonokoniosis.—When the particles are inhaled with the inspired air, the coarser particles are filtered by the vibrissæ at the entrance to the nostrils. The other particles are deposited throughout the respiratory tract, commencing from the nasal mucous membrane up to the alveoli of the lungs. The ciliated epithelium of the upper respiratory tract gradually expels the deposited particles by means of the action of the cilia and they are also removed by the mucous secretion and coughing, which mechanically act in expelling them. Those deposited in the alveoli are taken up by the leucocytes and the cells lining the alveoli, and carried through the lymphatics into the interalveolar septum, and ultimately into the lymph glands at the root of the lungs. During their passage through the alveolus, the particles produce a slight catarrh, resulting in an increase of expectoration and desquamation of the lining cells. In rare occasions, the bronchial glands are irritated to such an extent as to enlarge considerably, with ulceration and often evacuation into the blood vessels at the root of the lungs, and may be disseminated to distant parts of the body and are deposited in all organs.

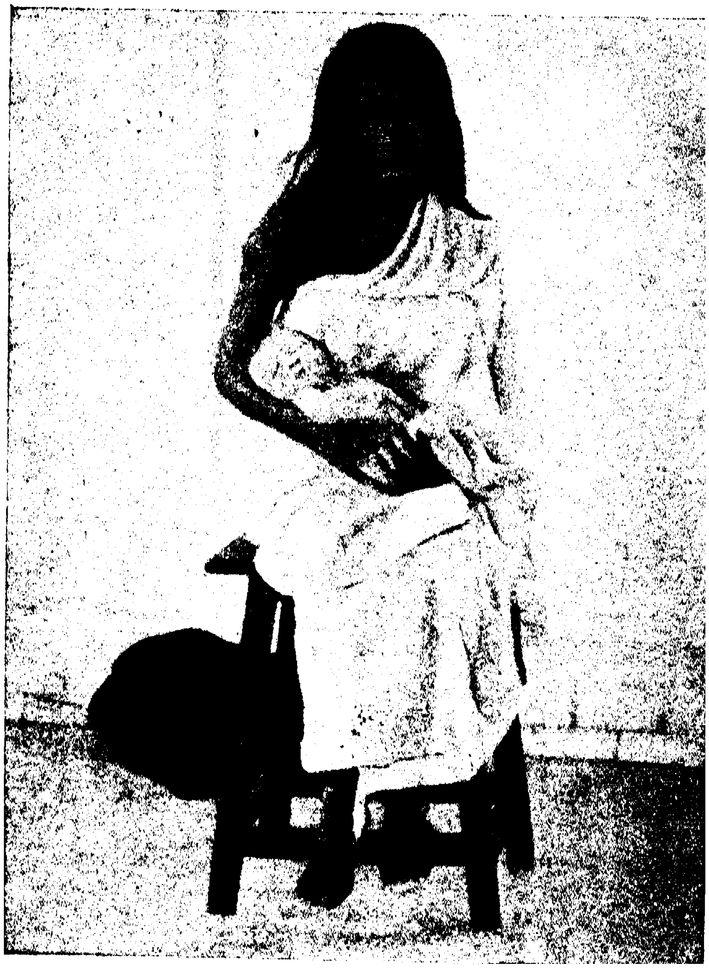


Fig. 296. Congenital albinism of the baby. (From Dr. Kedarnath Das's case.)

(2) *Tattoo marks.*—These are due to mechanical introduction of insoluble pigments into the skin by means of needle pricks.

(3) *Argyria.*—This depends on the deposition of silver salts (probably albuminates) into the skin, connective tissue, in the wall of the intestine, mesenteric glands, spleen, kidney, etc., due to long continued use of silver salts.

(4) Long continued use of *lead, bismuth, iron, etc.*, often causes deposition of pigments in the tissue,

Deficiency of the Normal Pigment of the Skin.—This is also of great importance in human pathology:—

(1) LEUCODERMA OR VITILIGO.—This is a localised absence of the pigment, occurring as white patches on the skin, often surrounded by denser pigmentation of the skin. The deficiency also extends to the hair, if there be any present in the patch. This also takes place in deep scar tissue, due to the deficiency of melanoblasts.

(2) ALBINISM.—This is characterised by a general lack of normal pigmentation and is usually congenital, and often found in several members of the same family. On account of the universal loss of the pigment, especially of the eyes, the patients cannot bear even ordinary light which is too dazzling for them. They also suffer from nervous disorders.

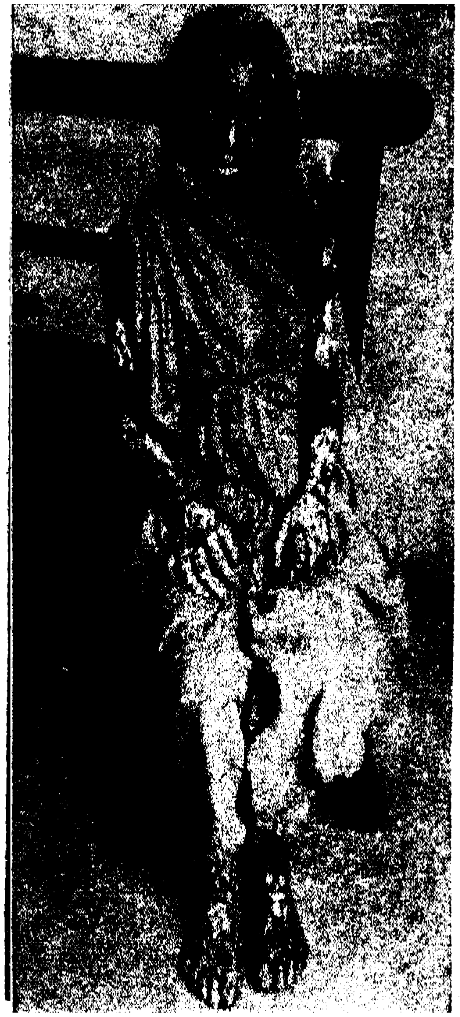


Fig. 297. Leucoderma.
(From Calcutta Medical Journal.)

NECROSIS AND GANGRENE

Necrosis is the death of the tissues still attached to the living body. The more rapidly the death occurs, the more unchanged is the cell form.

Putrefaction is the hydrolytic dissociation of dead protein, with production of gas and odour.

According to the extent of the death of the tissues, necrosis is divided into (1) *Necrosis*—the death of individual cells, and (2) *Gangrene*—the death of tissue *en masse.*

Necrobiosis.—(*a*) *Change of the cell body.*—(*i*) Change of the cell form and cell granules. The granules become bigger at first and are dissolved and expelled; (*ii*) Change in the staining characters.

(*b*) *Change of the nucleus.*—(*i*) Karyorrhexis—breaking down of the nucleus into granules; (*ii*) Karyolysis—solution of nuclear cromatin; (*iii*) Pyknosis—collection of chromatin into a few dense fragments which stain deeply.

Etiology.—Necrosis is generally due to malnutrition, which may be grouped as follows:—(1) Nutritional and circulatory disburbances, (2) Traumatic causes, (3) Toxic causes, and (4) Trophic disturbances.

All these factors, although quite distinct from one another, lead to the ultimate result, which are *the nutritional disturbances caused by them.* The **types of necrosis** caused by them are not identical in all instances and depend on the following:—(*a*) Resistance of the tissue, (*b*) Vitality of the tissue, (*c*) Temperature of the tissue, (*d*) Age of the individual, (*e*) Amount of circulation in the part, (*f*) Presence of other disease, (*g*) Virulence and activity of the causal agent, (*h*) Duration of the action of the causal agent.

Simple necrosis is that where the death is very rapid, *e.g.*, by fixation of tissue for histological purposes. The form and structural details remain unaltered.

(1) NUTRITIONAL AND CIRCULATORY DISTURBANCES.—These depend on—

(*i*) Obstruction in the artery.—The obstruction may be due to causes in the lumen, *e.g.*, thrombosis, embolism; causes in the wall, *e.g.*, rupture, arteriosclerosis with thickening of the wall and narrowing of the lumen, spasm of the vessel wall; or causes outside the wall, *e.g.*, ligature, compression by tumour, etc.

(*ii*) Obstruction in the vein.—This usually takes place in case of tight bandaging of a part, without interfering with the arterial circulation. In case of injury to both the artery and the corresponding vein, or the injury to the vein during ligation of the artery, gangrene usually takes place.

(*iii*) Obstruction to the capillaries.—This usually depends on the pressure on the capillaries, generally caused by pressure by new growth, extravasation of blood, or exudates from the blood or lymph vessels.

(*iv*) Cardiac weakness and other constitutional disorder, *e.g.*, anæmia, cachexia, senility, nephritis, diabetes, etc.

(2) TRAUMATIC CAUSES.—These may be either mechanical, thermal, electrical, or due to pressure. Mechanical injury in the form of direct or indirect violence causing necrosis is very common. Heat and

cold, when extreme, often lead to extensive necrosis. Irritant chemicals, long-continued irritation caused by X-rays or radium, are also destructive to the cells.

(3) TOXIC CAUSES.—These are very numerous. They may be—

(*i*) Chemical agents which generally affect the protein constituent of the cell, leading to coagulation of the cell protoplasm, *e.g.*, carbolic acid, corrosive sublimate, acids, alkalies, metallic salts, alkaloids.

(*ii*) Bacterial toxins are the commonest and form a very extensive group, *e.g*, the tubercle bacillus, causing necrosis of the centre of the tubercle, and diphtheritic affection of the throat.

(*iii*) Metabolic toxin, *e.g.*, diabetes, also forms a very important part in the causation of necrosis.

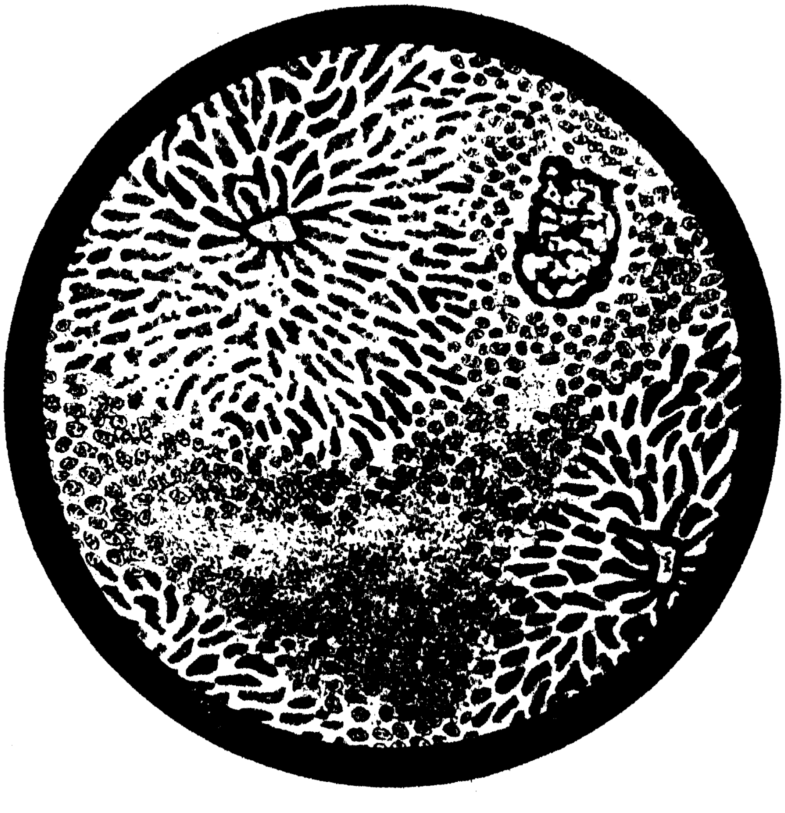


Fig. 298. Section of liver showing periportal necrosis and hæmorrhage in a case of *Eclampsia*

4. TROPHIC CAUSES.—These are illustrated in cases of bed-sore, arthropathies, perforating ulcer of the foot. The exact part played by trophic disturbances is not definitely known. Long continued pressure, or exposure of an anæsthetic surface to repeated injuries, probably plays the greater rôle.

Susceptibility of the Tissue Elements for Necrosis.—Different cells show great variation in their susceptibility to undergoing necrosis. The more specialised the cell, the earlier the necrosis. In infarction of the kidney, one notices at the margin of the infarct that the epithelial cells are all necrosed, whereas the connective-tissue cells are still living.

Varieties of Necrosis.—They are (1) Focal necrosis, (2) Coagulation necrosis, (3) Colliquation necrosis, (4) Caseation, (5) Autolysis, (6) Fat necrosis, (7) Hæmolysis, (8) Gangrene.

(1) *Focal Necrosis.*—This includes the necrosis affecting only a few cells in the organ and is very common in certain infectious diseases and intoxications, such as pneumonia, typhoid fever, eclampsia and other toxæmias of pregnancy. The liver is very frequently affected; certain groups of cells are affected, resulting in autolysis and death, which are subsequently removed by phagocytes and the area converted into a fibrous mass, on account of deposition of fibrous tissue.

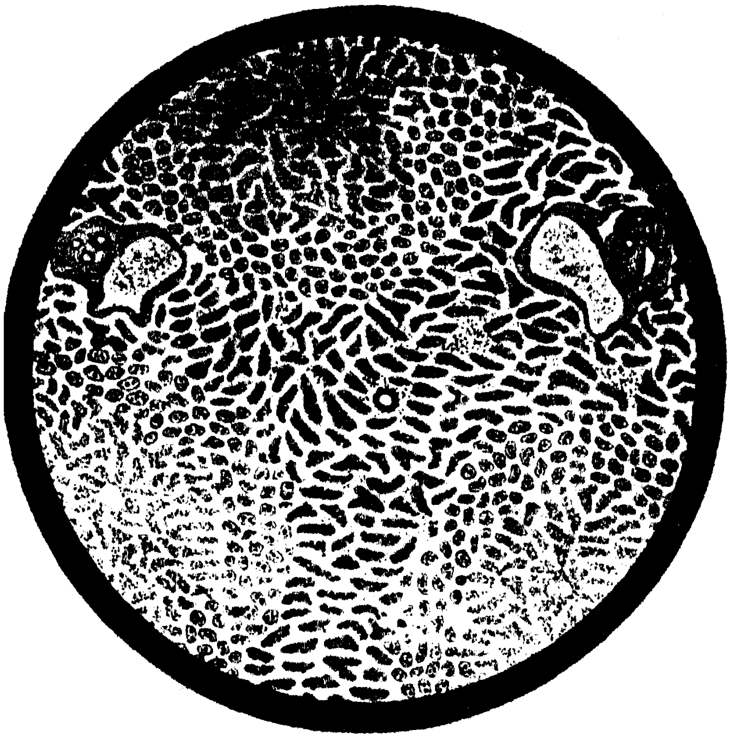


Fig. 299. Section showing changes of central necrosis in the liver in a case of *Pernicious Vomiting of Pregnancy.*

(2) *Coagulation Necrosis.*—This includes the necrosis of tissues in which the proteins undergo changes similar to those of coagulation, possibly due to the production of some ferments from the necrosis of cells. It is thus essential that the tissue rich in proteins will suffer most. The changes, although distinct chemically from the coagulation of the inflammatory exudate, are more or less identical physically. It is frequently seen in the serous and mucous membranes and also in the muscular tissue, specially the myocardium, and occurs in infection caused by the diphtheria bacillus, pneumococcus, B. dysenterie, B. tuberculosis and pyogenic organisms. Chemical and thermal irritants are also the causative factors. It usually depends on the production of substances (coagulins) by the necrosed cells, which unite with lymph, with, subsequently, coagulation and formation of pseudomembranes. Infarction of the kidney is another good example.

When examined with the naked eye, the tissue is seen to have an opaque, glairy and pale appearance, changing to grey membranous areas. Microscopically, the exudate is seen to consist of granular and fibrillar form of fibrin, which has been fixed to the tissues. The cells, including the red blood cells and leucocytes present in the area, do not take the stain properly. The nucleus may stain faintly in the earlier stages, later it disintegrates and disappears completely, the protoplasm of the cells being converted into a structureless hyaline material. The cells often show fatty degeneration, vacuolation, fragmentation and loss of intercellular material. Blood vessels at the margin are thrombosed. The termination of coagulation necrosis is by the process of ulceration and ultimately by the casting of the necrosed area. If the area is very small, it may be encysted and dissolved, and complete absorption may take place. In other instances, fibrosis results and the area is converted into a scar tissue.

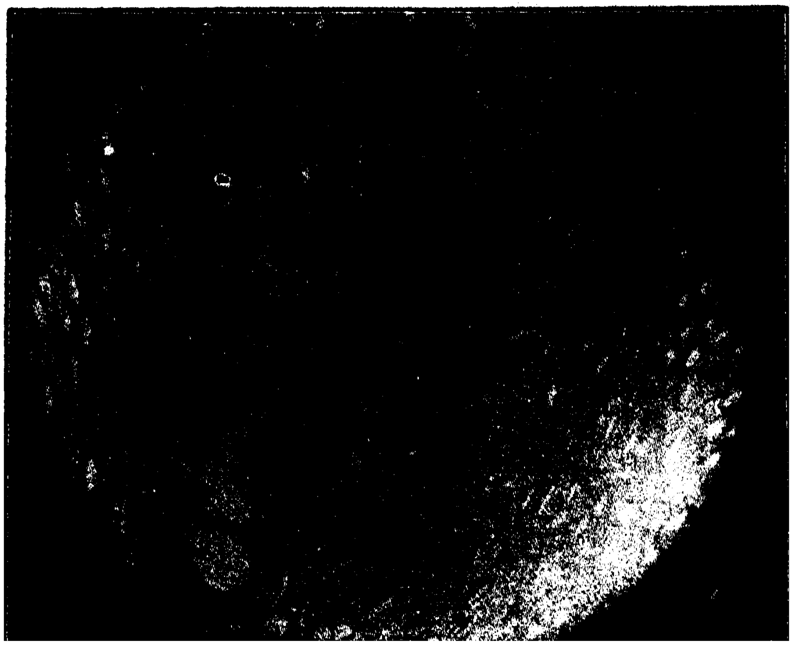


Fig. 300. Focal necrosis of the kidney.

(3) *Colliquation Necrosis.*—This consists of death, followed by liquefaction of the tissues. This may occur (*a*) primarily, as in suppuration, where all the structures involved, whether soft or hard, are liquefied by the action of the products of the bacteria and pus cells; (*b*) Secondarily, as when colliquation takes place in a tissue already necrosed or degenerated by other causes. This is illustrated in the liquefaction of the areas of coagulation necrosis, caseation, gangrene, etc.

A very common seat of this form of necrosis is the anæmic infarction of the brain following embolism. The tissue in the early stages is softer than normal and there is infiltration of fluid into it, resulting in solution of the tissue elements, with the formation of a semisolid mass. The cells are seen in all stages of degeneration (fatty and hyaline), which are subsequently dissolved by the enzymes of the bacteria in case of infective processes, or by autolytic ferments in case of non-infectious conditions. The necrotic matter is gradually absorbed, or liquefied with formation of a cyst (colliquation cyst). In some instances the contents may coagulate. The area may be of different colour, according to the presence of fat, or pigment (blood or bile).

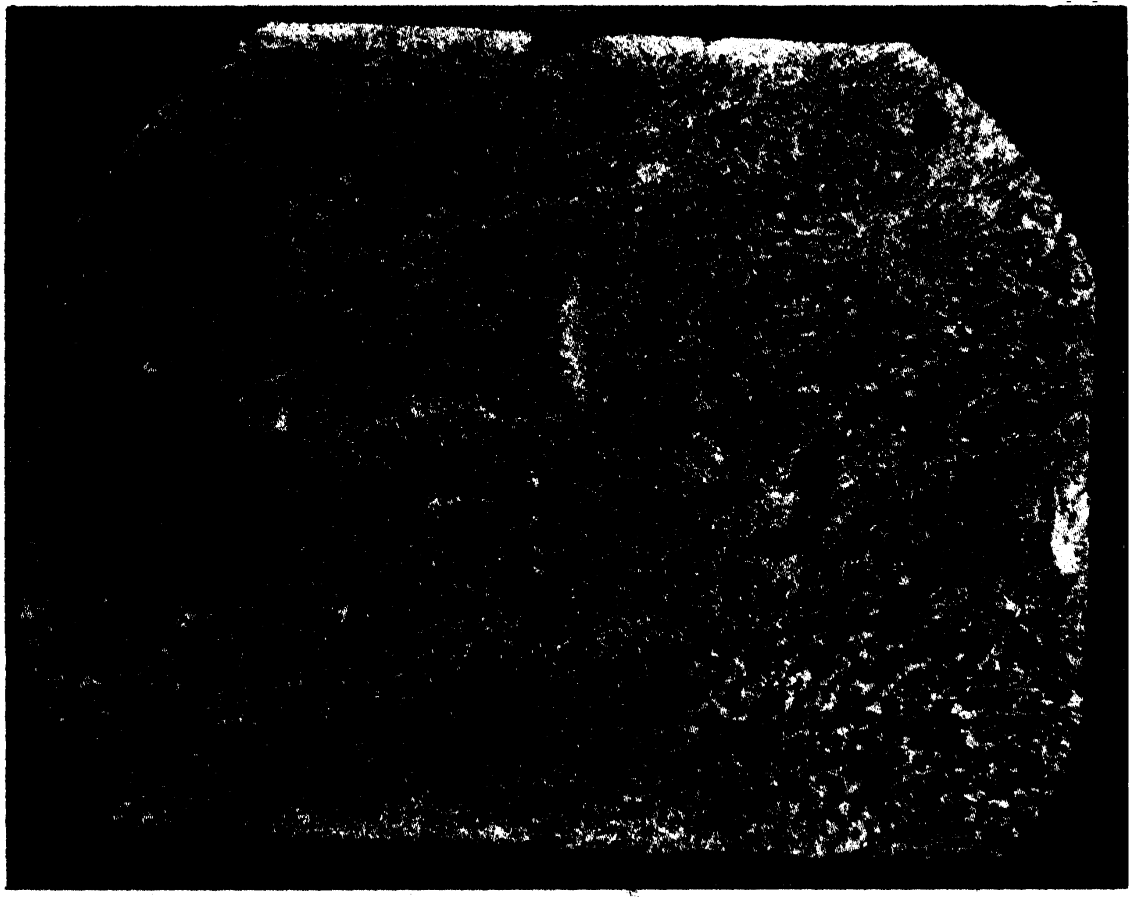


Fig. 301. Acute atrophy of the liver.

The termination takes place by absorption, coagulation, encystation or rarely by organisation.

(4) *Caseation.*—This consists in the formation of cheese-like masses (cheesy necrosis). This is most commonly found in cases of tuberculosis, but may also be found in other granulomas, especially syphilis (gumma).

It is essentially the same process as coagulation necrosis, and is believed to be due to the lack of accumulation of enzyme-bearing leucocytes to the area, and thus the necrosed area does not undergo liquefaction. The inhibitory action of the toxins of the bacteria on the activity of autolytic ferments, as well as the anæmia of the caseous area, due to diminished blood supply, also contribute to the formation of caseation.

The caseated material has an appearance of that of cheese and may be firm or soft. Microscopically, the cells show no differentiation, the whole of the area being converted into debris. The area does not take the stain. It is surrounded by a zone of round celled infiltration and other inflammatory changes.

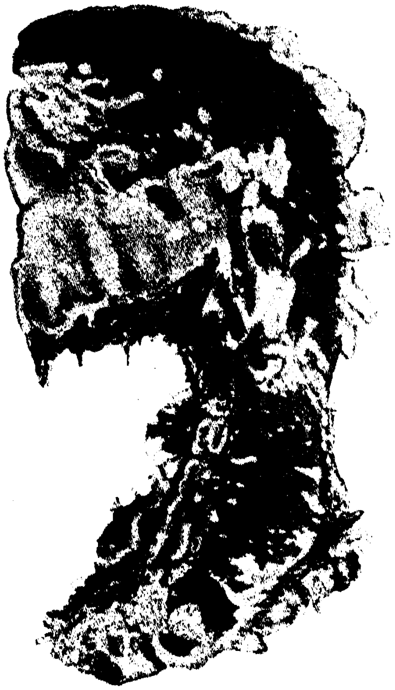


Fig. 302. Pancreas showing fat necrosis and hæmorrhage.

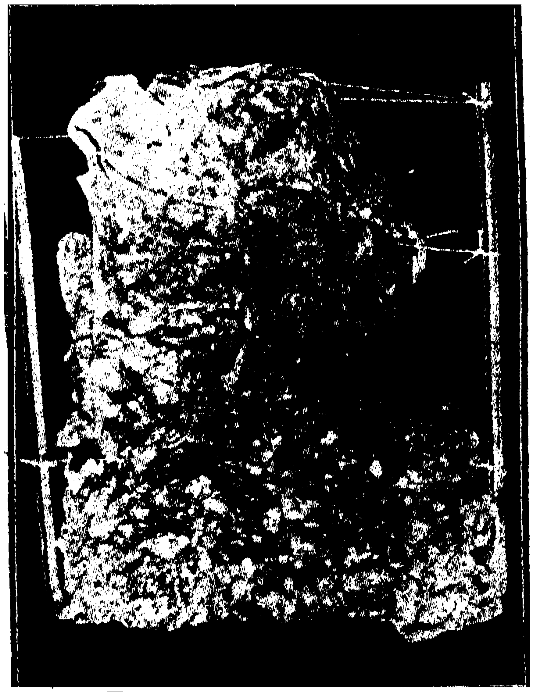


Fig. 303. Omentum showing fat necrosis.

(From Pathological Museum, Carmichael Medical College.)

The termination of caseation is generally by evacuation, either through some natural channel or by the surgeon. If very small, it may be absorbed, or encysted, and may remain encapsulated for a long period, with subsequent deposition of calcareous particles, and may be calcified.

(5) *Autolysis.*—This consists of softening and digesting the tissue elements by enzymes produced by themselves. These enzymes are generally derived from the dead cells, more specially the leucocytes.

The resolution of a pneumonic consolidation of the lungs, or softening of a thrombus takes place by means of autolytic process.

(6) *Fat Necrosis.*—Acute pancreatitis is the cause of extensive fat necrosis. The lesion appears mostly on the omentum, mesentery and retro-peritoneal tissue.

It consists in the formation of white, opaque, soft areas, generally due to the action of one of the ferments (lipase) of the pancreatic secretion. This generally happens when there is leakage of pancreatic secretion into the abdominal cavity, due to a defect in the pancreatic duct or disease of the pancreas. This is favoured by the entrance of sepsis, bile or intestinal contents into the pancreatic duct. Microscopically, fatty acid crystals and lime salts are found in abundance, with accumulation of leucocytes at the margin.

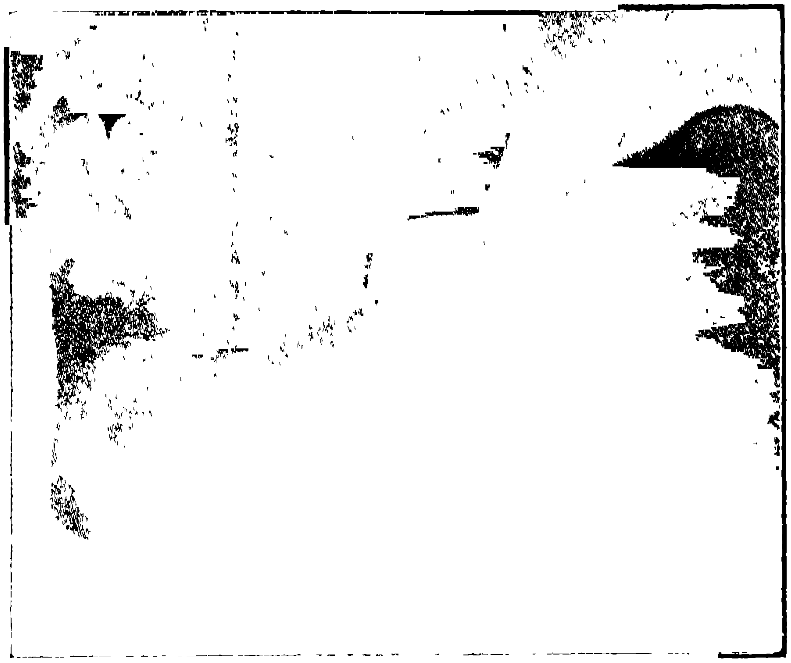


Fig. 304. Gangrene of the foot secondary to popliteal aneurism. (From Carmichael Medical College Hospital.)

(7) *Hæmolysis.*—This consists in the destruction of red blood cells, with liberation of free hæmoglobin into the circulation. (See under Pigmentary Infiltration.)

(8) *Gangrene.*—Gangrene is the death of tissue *en masse*, and is usually caused by bacteria. It may be (*a*) primary—when the gangrene is caused by a particular organism as its direct result (gas gangrene may be named as an instance), and (*b*) secondary—when the gangrene is caused by saprophytic bacteria, causing decomposition and putrefaction of areas already necrosed from other causes.

Secondary gangrene may be of two forms, *viz.*, (1) Dry gangrene, and (2) Moist gangrene.

Dry Gangrene or Mummification.—This occurs when the putrefactive processes are not marked or absent. It takes place when the dead tissue contains little moisture, when it is superficial, and can be acted upon by the air. The appearance of the area is usually dark and there is little absorption of toxin from the area. Examples:—

(*a*) *Mummification of the umbilical cord* after birth.

(*b*) *Senile gangrene* of the toes due to vascular disturbances.

(*c*) *Raynaud's disease*—this affects the peripheral parts of the body such as the toes, fingers, tip of nose, etc.

(*d*) *Frost bite,* due to freezing.

(*e*) *Trench foot,* due to damage to blood vessels by freezing.

(*f*) *Ergotism.*

(*g*) *Lathyrism.*

(*h*) Moist gangrene may terminate into dry form when putrefaction is slow and rapid evaporation of fluids takes place.

Moist Gangrene or Mortification.—This consists of putrefaction of the necrosed tissue by saprophytic organisms and takes place when the tissue is moist. Examples:—

(*a*) *Diabetic gangrene.*

(*b*) *Cancrum oris* or *noma.* This occurs in cases of kala-azar as a complication. In other countries it is commonly caused by B. diphtheria.

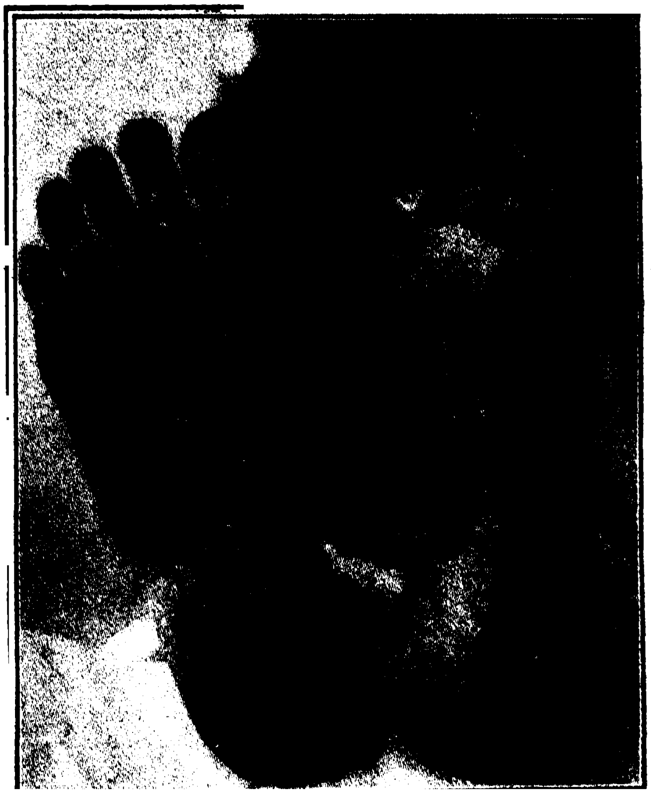


Fig. 305. Perforating ulcer of the foot (left) following neuritis. (From Dr. Bidhan Chandra Roy's case).

(*c*) *Hospital gangrene.* Before the days of Lister, virulent infection of wound used to take place after any operation, resulting in necrosis, putrefaction and high mortality.

(*d*) *Gangrene of the lung*—occurring secondarily from pneumonia, or embolism of the pulmonary vessels.

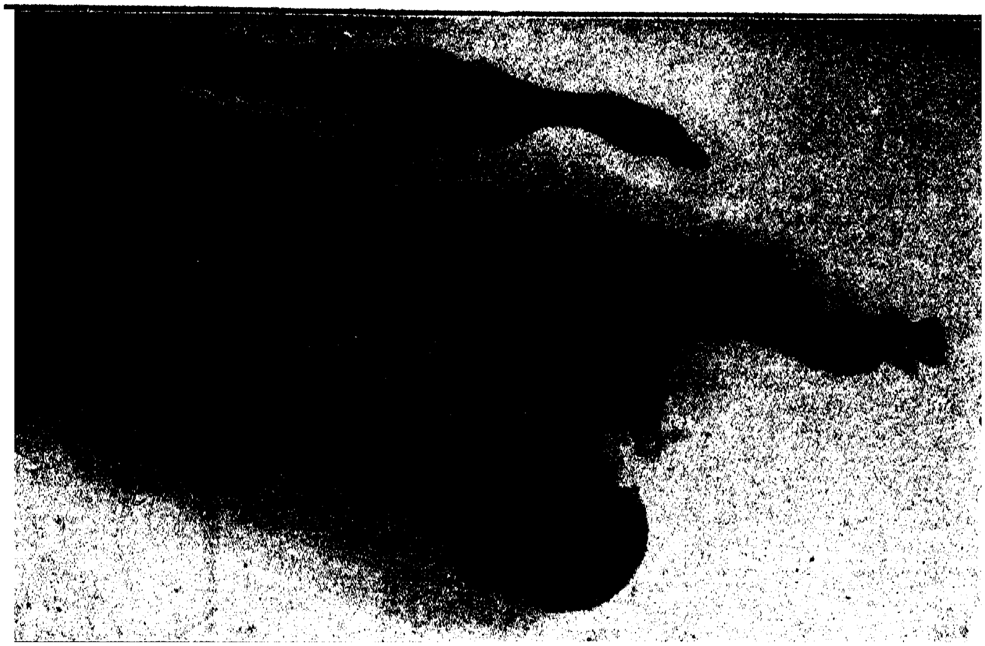


Fig. 306. Skiagram showing loss of phalanges following gangrene

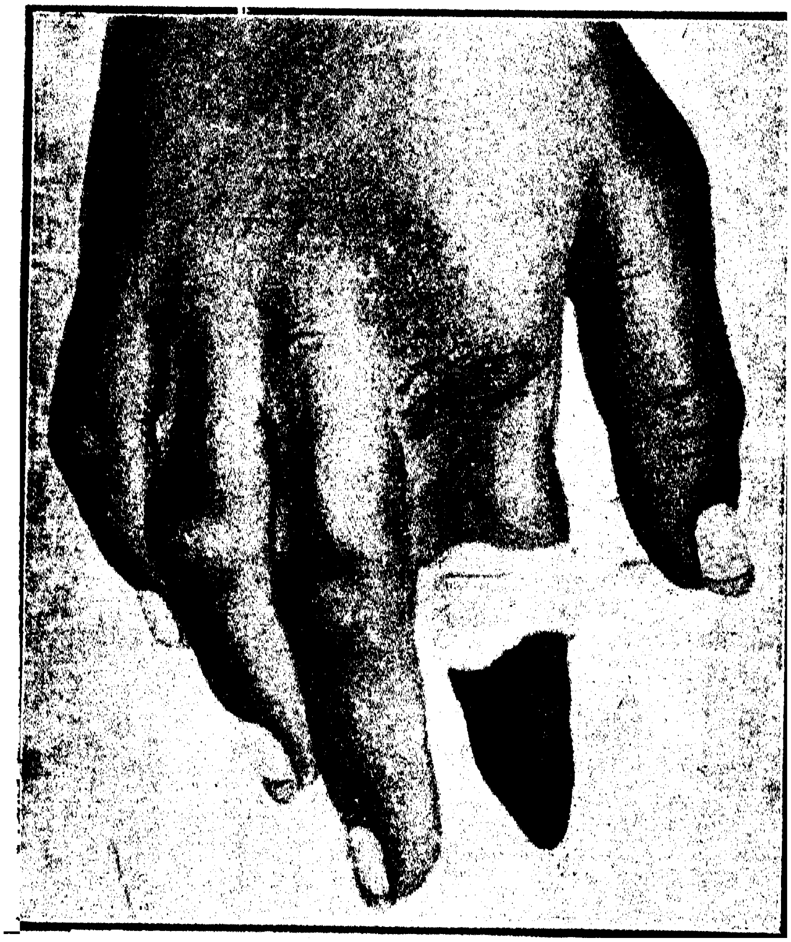


Fig. 307. Localised gangrene (index finger). (From the Path. Museum, Cal. Med. College).

(*e*) *Gangrene of the intestines*—occurring in cases of injury, strangulation or intussusception of the bowels, due to vascular disturbances.

(*f*) *Bed sores, myelitic cystitis*, etc., from trophic disturbances.

(*g*) *Gas gangrene or emphysematous gangrene* caused by B. œdematis maligni, B. ærogenis capsulatus, etc. This was fairly common during the Great War. This is usually caused by

B. Welchi. B. of malignant œdema (motile) and B. Welchi (non-motile) are saccharolytic organisms, and not proteolytic, whereas B. sporogenes (actively motile) is non-saccharolytic but proteolytic and hence putrefactive.

In the early spreading stage of gas gangrene, it is the saccharolytic organisms which are predominent, and they alone are responsible for the production of gas, which damages the tissues without causing putrefaction. In the later stage, the proteolytic putrefactive organisms, in themselves non-pathogenic, invade the already damaged and necrosed tissue, leading to gangrene.

Morbid Anatomy.—The areas are generally dark-coloured, slimy, soft and pulpy. The moist forms, on account of the putrefaction, give an offensive odour, with the formation

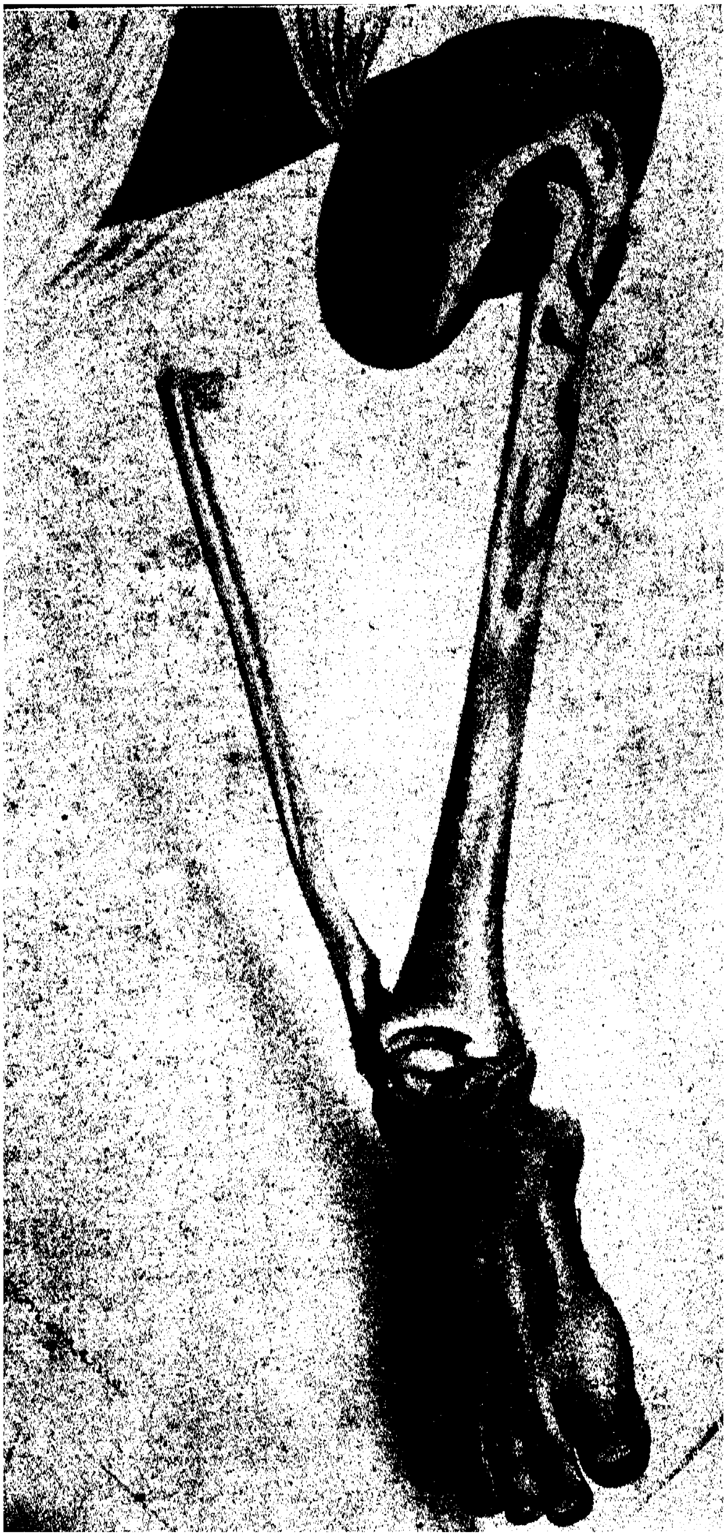


Fig. 308. Dry gangrene of the leg. (From the Path Museum, Cal. Med. Col.)

of gas and other decomposition products. These products are occasionally absorbed into the circulation, resulting in *sapræmia.* The dead tissue is known as *slough* or *sphacelus.*

Morbid Histology.—The cells are first to degenerate. The nucleus disappears, the protoplasm disintegrates, the cell is converted into a granular debris, and ultimately all trace of the structure of the cells is lost. The intercellular substance is also affected and degenerated, according to the density and resisting power. The ultimate result is the destruction of all the elements. Extensive hæmorrhages often take place, due to erosion of the blood vessels. Numerous crystals of fatty acids, cholesterin, pigments and other substances appear in the tissue, along with numerous other gaseous and other putrefactive products. The necrosed tissue is surounded by an area of inflammatory zone, specially near the healthy tissue.

Termination of Necrosis.—The presence of necrosed tissue excites reactions in the neighbouring tissues, leading to the accumulation of leucocytes, which produce proteolytic ferments which are responsible for gradual absorption of the necrotic tissue. Autolytic ferments may also serve the same purpose. When complete absorption is not possible, encapsulation by fibrous tissue takes place or calcification may subsequently occur.

(1) *Exfoliation of the Slough.*—Along with the putrefactive processes, the neighbouring healthy tissue is being constantly irritated, either by bacterial products or the products of putrefaction. Thus an inflammatory zone develops at the nearest healthy tissue and is known as the *line of demarcation.* This line of demarcation is of great importance form clinical point of view, as it indicates that the progress of the formation of gangrene has come to its limits and that Nature is attempting to separate the dead from the living tissue. The living tissue shows an area of ulceration at the line of demarcation which is called the *line of ulcertion.* Sooner or later, active process of repair commences, with formation of granulation tissue at the line of ulceration, resulting in complete separation of the necrosed area. Thus the slough separates naturally, without any surgical interference, but fails to do so completely on account of the bones present in the gangrenous limb. The bones remain protruding with all the soft tissue exfoliated. Surgical interference is necessary for the bony stump. In situations where bones are not generally involved, as in cancrum oris, natural exfoliation of the sphacelus may be complete.

(2) *Sequestration.*—This takes place in case of necrosis of bony tissue. Here regeneration of bone takes place almost simultaneously with the degenerative changes, so that, the dead bone is often surrounded by new healthy bone. The dead bone in this condition is known as the *sequestrum*. Complete recovery will be considerably delayed for an indefinite period, and multiple sinuses will result com-

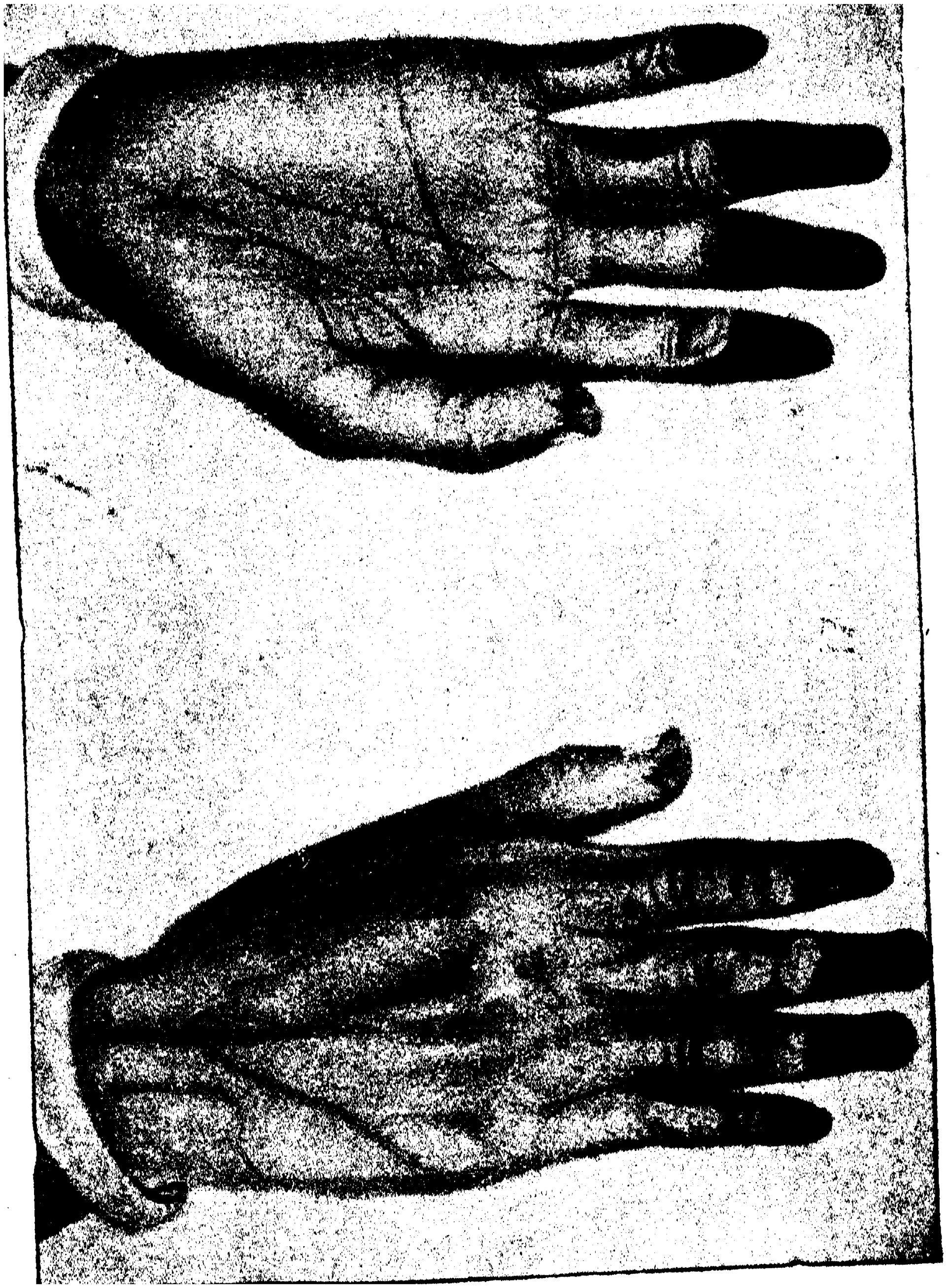


Fig. 309. Symmetrical gangrene.
(From the Path. Museum, Cal. Med. Col.)

municating externally, until the sequestrum is removed, either naturally or by surgical interference. The removal is followed by rapid healing.

(3) *Encapsulation.*—This takes place when the necrotic material is deep-seated and thus exfoliation of the same is impossible. When further degenerative changes cease to take place, fibrosis occurs in the surrounding tissues with complete encapsulation of the area. The fibrous tissue gradually contracts, resulting in the partial absorption of the necrotic material and occasionally a cyst forms, on account of colliquation of the material. This commonly takes place in the brain after embolism or thrombosis.

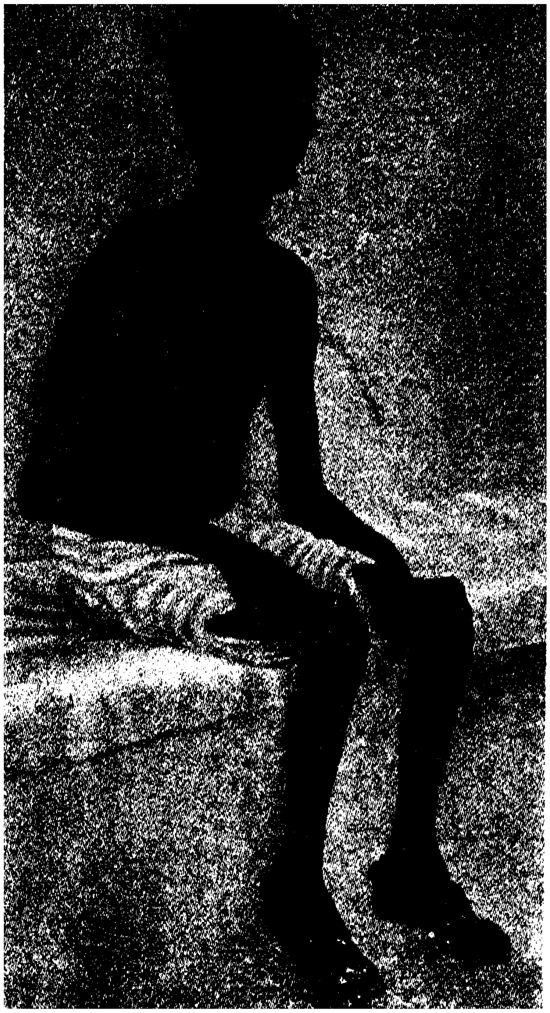


Fig. 309-A. Gout with tophi.
(From Dr. Jitendra Nath Moitra).

(4) *Organisation.*—In this condition fibrous tissue grows and ultimately replaces the necrotic area, resulting in the formation of a scar tissue.

(5) *Absorption.*—When the necrotic area is very small and the putrefactive process has ceased, absorption of the necrosed material takes place, due to the contraction of the fibrous tissue in the area.

(6) *Calcification.*—Calcareous particles are deposited when absorption and cicatrization of the necrotic tissue is delayed. This is commonly found in the caseation of tubercle, phleboliths, etc.

CHAPTER XXXIII

DISEASES OF THE BLOOD

General Considerations.—The primitive red blood cells originate from the endothelium of the capillaries formed by the angioblasts of the *area vasculosa* of the embryo. These primitive blood cells are basophilic megaloblasts. With gradual appearance of hæmoglobin the cells stain a mixture of blue and red forming what is known as polychromatophilia. This basophilic substance appears as fine reticulum when stained supravitally with brilliant cresyl blue and thus their presence indicates the youth of the red cell. These young erythrocytes are known as reticulocytes and form about 0.5-1 % of normal erythrocytes of human blood. When this basophilic substance appears in the form of masses of granules, a condition of punctate basophilia, granular degeneration of the erythrocytes, basophilic stippling, etc., takes place.

In extrauterine life the white cells of the blood consists mainly of granular cells, neutrophiles (polymorphonuclears), eosinophiles and the basophiles. Each of them originates from a parent cell, the myelocyte, normally present in the marrow. The myelocyte is a cell larger than the polymorphonuclears with a large amount of cytoplasm containing a single lobed nucleus. The granules are fine and take the neutral, acid or the basic stain corresponding to the nature of the daughter cell.

The myelocyte, in its turn, is derived from a cell, premyelocyte, containing only a very few granules in the cytoplasm. The most primitive cell from which the premyelocyte originates, is the non-granular stem cell called myeloblast.

There are two views regarding the genesis of the white blood cells from this primitive stem cell, the myeloblast. The advocates of the dualistic view believe this to be the parent cell only of the granular white cells of the blood, the lymphocyte series having no relation to it. The uniterian school advocates that myeloblast is the primitive stem cell which differentiates into two groups, the granular (myelocyte series) and the non-granular (lymphocyte series).

PERNICIOUS ANÆMIA

The first description of pernicious anæmia has been given by Addison in 1855.

Etiology.—Some form of toxin is held to be responsible for the affection characterised by interference of erythrogenesis in the bone marrow. The nature of the toxin is unknown but may be of the nature of hæmolytic streptococcus, B. welchii or helminthes, *e.g.*, the ankylostoma or the bothriocephalus latus. It is apparent that the toxin irritates the marrow to an abnormal activity, resulting in the liberation of immature red cells in the circulation. The hæmolysis of these cells produces wide spread deposits of iron in the tissues. "The causative factor in pernicious anemia has been established as an intrinsic specific factor arising in the gastric mucosa and an extrinsic factor, probably a protein. It is, in effect, a type of deficiency disease."

Blood Changes.—In pernicious anæmia all the formed elements of the blood—erythrocytes, leucocytes and the blood platelets—show decrease in number. Of all these elements, the erythrocytes present the most constant and characteristic changes. The blood picture generally shows itself in the form of fœtal blood. Thus, macrocytes, basophilic stippling of the red cells, normoblasts, megaloblasts, polychromatophilia, etc.—the normal constituents of fœtal blood picture—are present in pernicious anæmia.

Changes of Erythrocytes.—SIZE.—There is a great variation in the size of the red blood cells, of which cells larger than normal—macrocytes or megalocytes form the main feature. Cells smaller than normal—microcytes may also be found. The variation in the size is known as anisocytosis. NUMBER.—The number of red blood cells is greatly reduced. A count below 1,500,000 is characteristic of pernicious anæmia. SHAPE.—The shape also varies greatly. The circular outline is early lost, being replaced by various peculiar forms. This variation of the shape of red blood cells is known as poikilocytosis.

Hæmoglobin Content.—As on the average, the red cells are larger than normal, and as they are well supplied with hæmoglobin, the colour index naturally becomes high. In acute cases the colour index is generally 1.2 or 1.3. But, as the total number of the red cells are greatly diminished, the total amount of hæmoglobin is necessarily low.

Nucleated Red Cells.—These are very common findings but may often be absent. The common form is the megaloblast, while normoblast and microblast may often be found. The normoblast appears in a large number during 'blood crisis.' CYTOPLASM.—This may be normal but very often shows polychromatophilia or even basophilia. RETICULOCYTES.—These are generally increased and their presence serves as an index of the regenerative activity of the bone marrow. A rise of 5 % or more of the total red cells (normal being about 1 %) indicates good response on the part of the bone marrow, while a low count always means a bad prognosis. The reticulocytes are absent in aplastic anæmia.

Leucocytes.—These are also diminished in number. The polymorphonuclears are mainly affected. Thus in the differential count the number of polymorphonuclears are found to be diminished, but there is a relative increase of lymphocytes as they are not diminished proportionately. A few myelocytes may also be present.

Blood Platelets.—They are diminished to a considerable extent and in severe cases may be found to be totally absent.

Serum.—The bilirubin content of the serum is increased and the Van den Bergh test gives an indirect reaction.

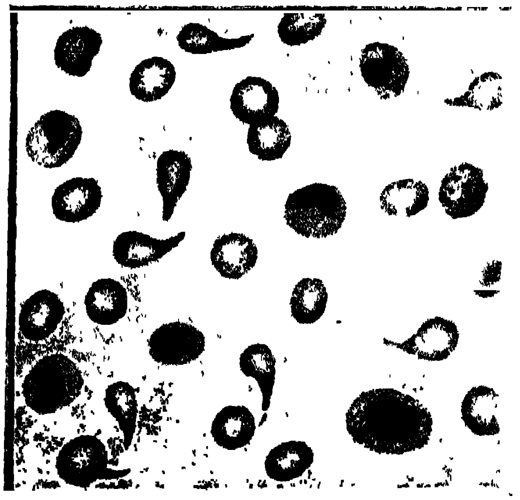


Fig. 310. Blood film of pernicious anæmia.

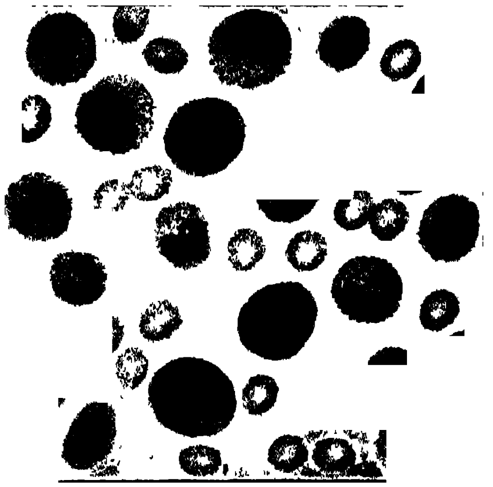


Fig. 311. Blood film of leukæmia.

Post-mortem Appearance.—The body is generally well-nourished. The skin and the fat appear lemon-yellow and the muscles a deep-red colour. The heart muscles appear pale, owing to fatty changes. Petechial hæmorrhages may be present in the serous membranes. The liver is fatty and shows presence of hæmosiderin by Prussian blue reaction. The spleen is greatly enlarged and shows deposits of hæmosiderin. The tongue shows chronic glossitis and the stomach an atrophic gastritis. This is responsible for the presence of *achylia gastrica* so common and prominent a feature in pernicious anæmia. The degenerative changes of the spinal cord affecting specially the posterior and occasionally the lateral columns are of special interest. The lesion is present in the form of numerous patches of degeneration, mainly in the upper part of the cord.

The main changes are observed in the bone marrow. The yellow marrow gradually changes into deep and red marrow. The red marrow shows great hyperplasia. Microscopically, the bone marrow presents a large number of megaloblasts. The polymorphonuclear myelocytes and megakaryocytes are greatly reduced in number. Large quantity of iron is found in the reticulo-endothelial apparatus of the bone marrow.

APLASTIC ANÆMIA

This is due to a cessation of the function of the bone marrow, generally caused by some toxin. The toxin may be of a chemical nature, *e.g.*, benzol; physical, *e.g.*, Roentgen rays; bacterial, *e.g.*, streptococcus or the end stage of pernicious anæmia. The blood picture in aplastic anæmia is characterised by diminution in the number of red blood cells, leucocytes and blood platelets, but the great variety of changes of red blood cells which are found in pernicious anæmia are generally absent.

SICKLE-CELL ANÆMIA

This is a congenital affection and is generally found amongst the Negroes. The blood picture is characterised by anæmia, and presence of large number of sickle or crescentic form of red blood cell and leucocytosis.

CHLOROSIS

This is an affection of the female sex, usually appearing at the time of puberty. The blood picture shows normal or slightly decreased number of red blood cells but a great deficiency of hæmoglobin. The colour index is therefore considerably low. The etiology is unknown.

LEUKÆMIA

This is a disease characterised by an enormous increase in the number of the white blood cells.

Etiology.—The etiology of leukæmia is unknown. No organism has been obtained from the tissue or from the blood.

A great deal of controversy exists regarding the nature of leukæmia. Some regard this to be inflammatory in nature, but the general view is that it is a form of neoplasm affecting the myeloid or the lymphatic tissue. The points in favour of neoplasm are the following:—(1) The steady progress invariably leading to a fatal termination. (2) The presence of the kind of cell in the tissue. (3) Occasional association of lymphosarcoma and leukæmia. (4) The acute leukæmia may be regarded as a malignant growth, while the chronic form only a simple tumour.

Varieties.—Leukæmia is generally distinguished under four different forms:—(1) Chronic myelogenous leukæmia or Splenomedullary leukæmia, (2) Chronic lymphatic or lymphoid leukæmia or lymphocythæmia, (3) Mixed form (myelogenous and lymphatic), and (4) Acute leukæmia.

Chronic Myelogenous Leukæmia

Blood Changes.—The blood picture is characteristic in leukæmia. The number of white cells is enormously increased with an average count of about 300,000, but it may go as high as 1,000,000. The white cells may even outnumber the red blood cells. The chief increase is mainly due to the appearance of granular leucocytes chiefly polymorphonuclears or the stem cell, the myelocyte. The polymorphonuclears may vary greatly in size. In some cases the eosinophiles may be found as the prominent cell. The myelocytes are mainly of finely granular neutrophiles and may constitute 50 per cent. or more of the leucocytes. The most primitive cell, the myeloblast, which is non-granular, may also be found, but this forms the principal type of cell in acute leukæmia. The myeloblasts are often mistaken for large lymphoblasts, but can be differentiated from them by the oxydase reaction. A positive oxydase reaction will show that they belong to the myeloid series and not to the lymphocyte series.

The total number of lymphocytes is increased but their percentage is markedly diminished. The red cells are greatly diminished, with considerable lowering of the colour index. Erythroblasts, mainly normoblasts are present. The blood platelets are usually greatly increased and may go up to 2,000,000 (normal being about 300,000).

The number of the white cells may be considerably reduced by means of Roentgen ray treatment, often coming down to normal, but still the abnormal forms esepcially the myelocytes generally persist.

Bone Marrow.—This is characterised by hyperplasia of the myeloid tissue. The bone marrow shows a gross leucoblastic reaction, The normal fat marrow of the long bones is replaced by greyish cellular marrow. It may occasionally be red or even purulent. A great absorption of bone trabeculæ may take place. This leucoblastic reaction may also occur in patches.

Histologically, the marrow shows enormous hyperplasia of the myeloid cells. Finely granular myelocyte and the non-granular stem cell, the myeloblast, are the main types of cells present. Granular neutrophilic polymorphonuclears, eosinophiles and basophiles are also present. The myeloblasts may occur in the form of islands surrounded by masses of myelocytes. Megakaryocytes are also increased, resulting in the increase in the number of blood platelets in the circulation. The leucoblastic reaction is so profound that occasionally the bone marrow is seen to be deficient in erythroblastic tissue, resulting in the very great increase in the number of the white cells in the blood accompanied by a severe form of anæmia.

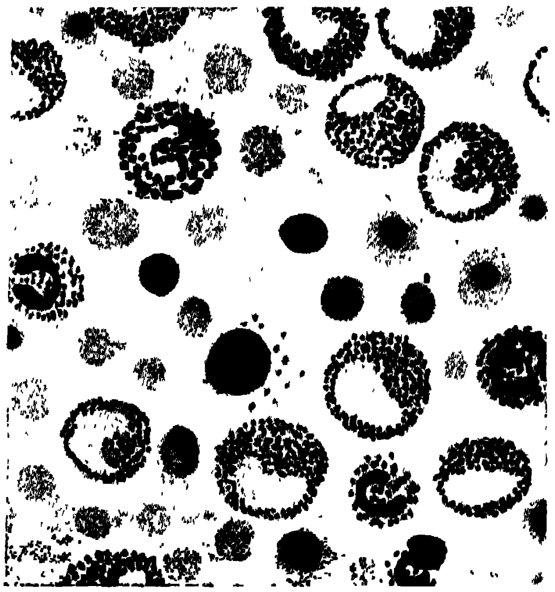


Fig. 312. Myelogenous Leukæmia.

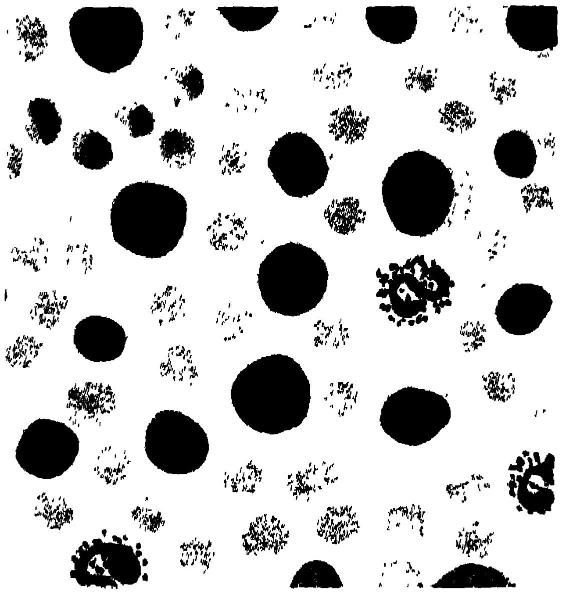


Fig. 313. Lymphatic Leukæmia.

Spleen.—The spleen is invariably greatly enlarged, hence the term *splenomedullary leukæmia.* It may reach well below the umbilicus but the general form is usually maintained. The capsule often shows evidence of hæmorrhage, either recent or old. On section, it appears dark red in colour and of firm consistency. *Histologically,* the lymphoid elements are seen to have been replaced by means of myeloid cells, thus giving the appearance of the bone marrow.

Liver.—The liver may be enlarged with evidence of myeloid reaction microscopically. The kidneys, heart, lungs, lymph nodes all show presence of myeloid reaction in them.

Chronic Lymphatic Leukæmia

Blood.—The total number of the white cells are greatly increased but not to such an extent as the myelogenous variety. The average count is about 100,000. The lymphocyte form about 90 per cent. or even 95 per cent. of all white cells. They are generally small lymphocytes and may not resemble the usual forms present in normal blood. The polymorphonuclear and other granular leucocytes are necessarily very few in number. The red cells are also diminished to a considerable extent with presence of a few or no normoblasts. The blood platelets are diminished in number.

Bone Marrow.—*Macroscopically*, the appearance is similar to that of myelogenous leukæmia. *Microscopically*, the lymphocytes are found to have replaced the myeloid tissue to a considerable extent.

Lymph Nodes.—These are enlarged throughout the body and may be of considerable size. *Histologically*, the gland structure is found to have been replaced by diffuse masses of lymphocytes.

Spleen.—This may be enlarged and shows hyperplasia of the lymph follicles. Other lymphoid tissue of the body, *e.g.*, the tonsils, Peyers patches and solitary follicles of the intestines, etc., also show similar changes.

Mixed Leukæmia

In this form the blood picture and the changes in other organs show evidence of lesion common to both the myelogenous and the lymphatic type.

Acute Leukæmia

This affection is very misleading on account of the suddenness and severity of the process. The leucocyte count may reach 100,000 within a few days, accompanied by extensive hæmorrhages and fatal termination. Although some cases show a lymphatic type of lesion, still in majority of instances it belongs to the myelogenous type.

Aleukæmic Leukæmia

This is a condition in which the patient shows all evidences of leukæmia both clinically and pathologically, except in the increase in the number of the white cells of the blood.

CHAPTER XXXIV

DISEASES OF THE CIRCULATORY SYSTEM

PERICARDITIS

Etiology.—*Primary.*—(1) Traumatic, (2) Tuberculous, (3) Pneumococcal; *Secondary* to—(1) Acute infectious fevers, *e.g.*, pneumonia, small-pox, typhoid fever, diphtheria, measles, rheumatic fever (rare in this country), etc., (2) Tuberculosis of the serous membranes, (3) Septic process in any part of the body, *e.g.*, puerperal septicæmia, pyæmia, etc., (4) Constitutional diseases, the so-called terminal complications, *e.g.*, in nephritis, gout, diabetes, arteriosclerosis, etc., (5) Extension of inflammation from the surrounding organs, *e.g.*, (*a*) Extension from without—pneumonia, pleurisy, inflammation of the mediastinal glands, ribs, vertebræ, rupture of subphrenic abscess, bursting of liver abscess into the pericardium, (*b*) Extension from within the heart.—ulcerative endocarditis, purulent myocarditis, etc.

Morbid Anatomy.—In the *early stage* the pericardium becomes hyperæmic, lustreless, and slightly rough. As the *stage advances* exudation takes place on the membrane, the exudation being rapidly changed into fibrin with no accumulation of fluid, forming what is known as the *dry or fibrinous pericarditis.*

The fibrin is deposited in considerable amount and on account of the constant movement of the heart and with it the pericardial surfaces, the appearance of the fibrin is modified as follows:—(*a*) A ridge-like appearance; (*b*) Honey-combed appearance; (*c*) Appearance resembling buttered surfaces of bread which have been drawn apart—the so-called *bread-and-butter pericardium;* (*d*) Fibrin rolled out into hanging membranes or in long shreds, giving the heart a shaggy appearance (*cor villosum*).

Pericarditis with effusion.—When the fibrin is deposited to a certain extent the exudation collects in the sac in the form of fluid serum. The effusion may be:—(*a*) Sero-fibrinous, *e.g.*, in pneumococcal pericarditis; (*b*) purulent *e.g.*, is cases of infection with pyogenic organisms or due to rupture of an abscess into the pericardium; (*c*) Hæmorrhagic, *e.g.*, in purpura, hæmophilia, tuberculosis, malignant growth of the pericardium, etc.

Morbid Histology.—(1) Engorgement of the pericardial vessels. (2) Fine fibrils of fibrin seen between the serosa cells. The fibrin is found in different layers, the fresh ones being finer while the old ones more massive and homogeneous. A large number of leucocytes are found in the fibrin layers. (3) Various degenerative and reparative changes of the serosa cells. (4) Presence of the following cellular elements in the effusion—(*a*) Polymorphonuclear leucocytes, (*b*) Mononuclears, if due to tuberculosis, (*c*) Plasma cells, (*d*) Serosa cells in all grades of changes, (*e*) Connective tissue cells. (5) The underlying myocardium shows much cellular infiltration and also appears œdematous. (6) When the pericarditis is of some standing, organisation of fibrin in various stages is seen. In addition to the various cellular elements, one finds young fibroblasts and large number of newly formed capillaries.

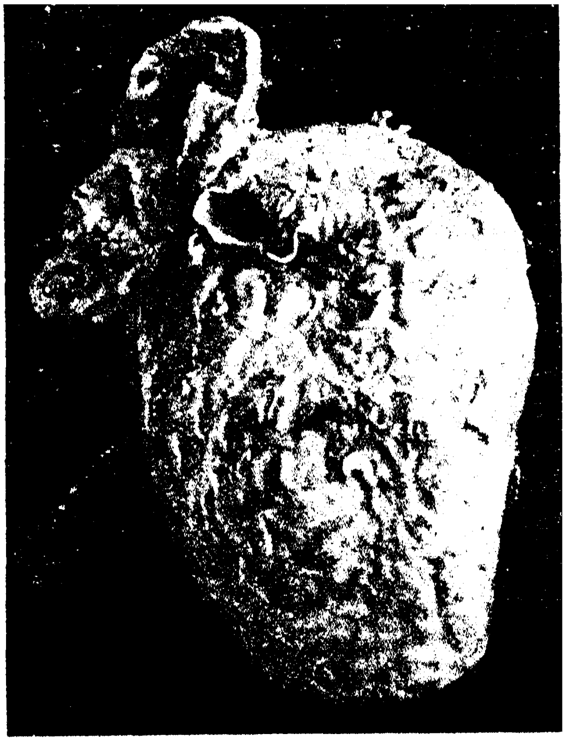


Fig. 314. Acute pericarditis, showing the shaggy appearance of the pericardium due to the deposition of large amount of fibrinous exudate. (From Path. Museum Carmichæl Med. Col.).

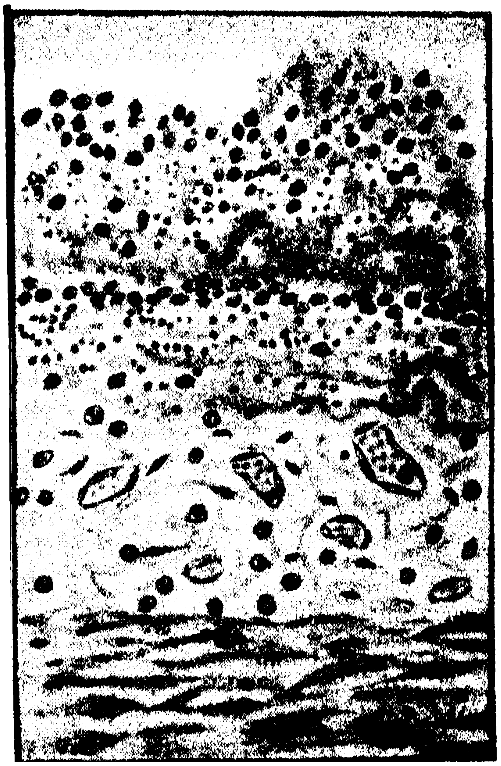


Fig. 315. Acute pericarditis.

Morbid Physiology.—The disturbance of the function is manifested in the form of the following clinical features which are commonly due to the factors mentioned against each:—*Pain*—Painful afferent stimuli are produced by rubbing of the inflamed surfaces. *Fever.*—A general manifestation of the inflammatory process. *Dyspnœa.*—Marked in pericarditis with effusion. This is due to (*a*) Less blood in lesser circulation as the vena cavæ cannot empty themselves into the right auricle; (*b*) Compression of the left lung. *Distension of the cervical veins.*—Due to the stasis of blood in the vena cavæ on account of the venous pressure being less than the fluid pressure within the pericardial sac. *Dysphagia, aphonia, irritative cough.*—These are due to the pressure on the œsophagus, trachea and recurrent laryngeal nerves respectively. *Low blood pressure, delirium, coma, etc.*—These are due to the defective blood circulation on account of less fluid in the arteries due to stasis in the vena cavæ and diminished force of the contraction of the heart.

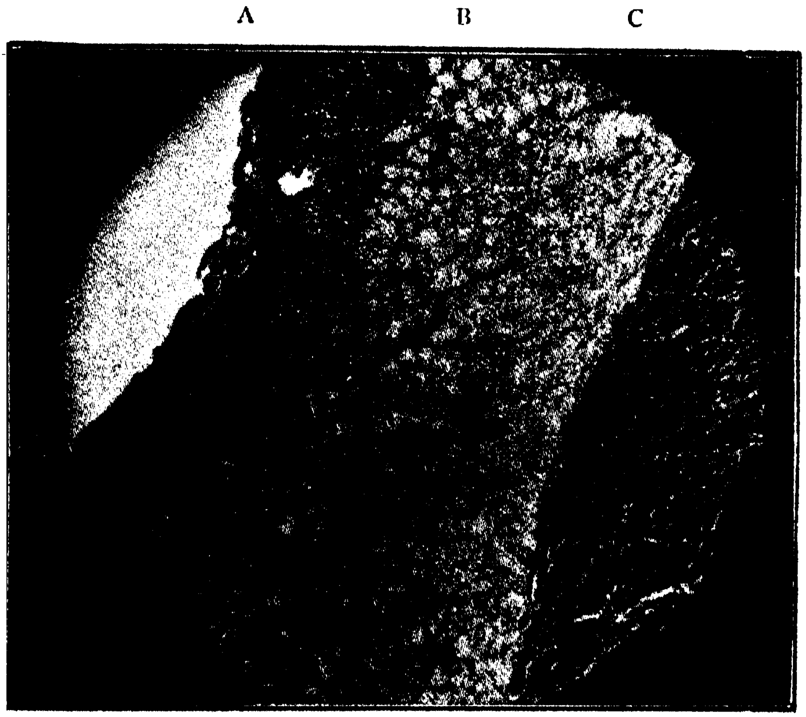


Fig. 316. Acute pericarditis showing (*a*) Great thickening of the pericardium, (*b*) Subpericardial fatty layer, (*c*) Heart muscle.

Certain facts peculiar to Pericardial Effusion.

Rotch's Sign.—The cardio-hepatic angle is dull on percussion as the space is being occupied by the effusion on account of its occupying the most dependant part of the pericardial sac. Bamberger sign—dullness and tubular breathing over the left scapular region—this is due to the compression of the left lung by the fluid in the pericardium, the pressure can be partly relieved on leaning forward and so the signs may also be partly obliterated.

Certain facts peculiar to Adhesive Pericarditis.

(*a*) Pulsus paradoxus—due to defective filling of the heart during inspiration, when the intrathoracic pressure is diminished, the fluid pressure in the pericardium over-weighs the venous pressure hence diminished flow of blood into the right heart—weak pulse during inspiration.

(*b*) Brodbent's sign--the pericardium is attached to the central tendon of the diaphargm which again is attached to the ribs from the sixth to the last. On account of adhesion between the two layers of the pericardium the central tendon of the diaphragm is drawn during each systole which is manifested by the retraction of the last ribs. The last two ribs being floating ones are most influenced by the retraction.

(*c*) Friedrich's sign—diastolic collapse of the cervical veins.

Termination.—(*a*) *Resolution.*—If exudate is scanty or the layer of fibrin is thin, complete absorption of the products may take place; (*b*) *Organisation.*—If the deposition of the fibrin is considerable, it is gradually been replaced by fibrous tissue resulting in thickening of the pericardium. In some instances the fibrous tissue grows and communicates with both the parietal and visceral layers leading to (*c*) *Adhesion.*—Of the two layers.

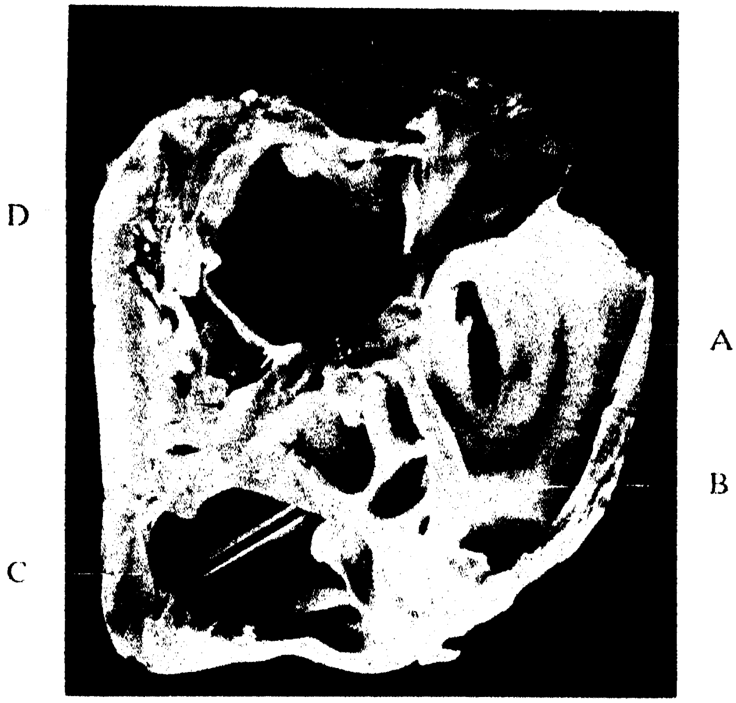


Fig. 317. Chronic endocarditis of the mitral valve with thickening and narrowing of the orifice—mitral stenosis. (*a*) Mitral orifice (thickened and narrowed), (*b*) Aortic orifice, (*c*) Pulmonary orifice, (*d*) Tricuspid orifice. (From Path Museum Carmi. Med. College).

ENDOCARDITIS

Normal Histology.—The endocardium is a membrane lining the cavities of the heart and also the papillary muscles. It is duplicated in places forming the valves. The endocardium consists of fibroelastic structures and covered by flat serosa cells (epithelium or endothelium). The valves contain abundant elastic fibres and few plane muscle fibres. The terminal branch of the coronary artery lies in the deep layers, and the endocardium itself gets its nutrition from the circulating blood in the chamber of the heart. In fætal life the vessel runs up to the margin of the valve.

Etiology.—(1) *Primary.*—Rare. (2) *Secondary* to—(*a*) Pneumonia (mitral affections), (*b*) Syphilis (aortic affections), (*c*) Rheumatic fever (mitral affections specially in Europe), (*d*) Other causes same as those for pericarditis.

Endocarditis generally takes place of the valvular endocardium. Of the different valves, the mitral one suffers very commonly, being about 60 per cent. of all cases of endocarditis, then the aortic (10 per cent.) and least frequent are the affections of the tricuspid and the pulmonary valves. Affections of the mitral and aortic valves together takes place in about 20-30 per cent. of cases.

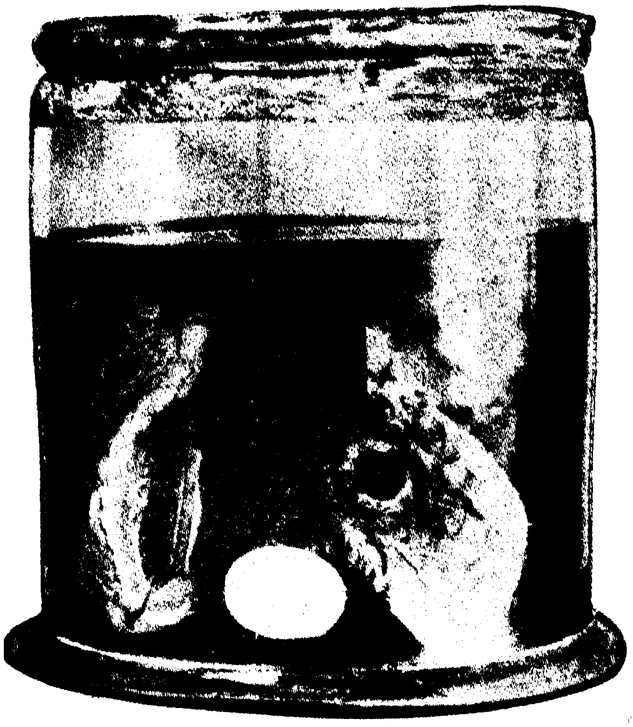


Fig. 318. Mitral stenosis—showing the ring-like mitral orifice.
(From Path. Museum, Cal. Med. Col.).

Morbid Anatomy.—(*a*) Inflammation of the valves—*valvular endocarditis;* (*b*) Inflammation of the lining membrane of the cavity of the heart—*mural endocarditis.*

Theories regarding the Mode of Infection of the Valves.

(*a*) The specific organisms circulate through the blood and directly impinge on the valves and cause lesions. This seems to be more frequent as is evidenced from the study of the normal histology of the endocardium.

(*b*) The organisms pass through the coronary vessels, block up the endarteries at the base of the valves (embolic process) and there start the lesions.

The Changes.—(1) Hyperæmia of the valves, as a result of the inflammation. (2) The valves become swollen and translucent at or near their free margins—irregular beaded appearance. (3) Formation of vegetations on the cusps of the valves: small masses, at first papillary in character, later vegetations enlarge, become opaque, of whitish or yellowish-white in colour, in some cases attaining a considerable size.

Varieties.—Endocarditis may be acute or chronic. Acute endocarditis is generally seen in either of two forms:—(*a*) Verrucous thromboendocarditis (simple endocarditis); (*b*) Ulcerative thromboendocarditis (ulcerative or malignant endocarditis).

The verrucous thromboendocarditis is the form very commonly seen The valve presents a rough, wart-like deposit on the surface. This wart-like deposition (*vegetation*) is composed of elements comprising a thrombus, *e.g.*, abundance of blood platelets mixed with fibrin, leucocytes and red blood cells. The fixed tissue cells of the valve proliferate subsequently and outgrowth of newly formed blood vessels takes place. Fibrosis is a late manifestation and is due to the organization of the inflammatory products (*chronic endocarditis*) Fresh endocarditic deposits may occasionally be seen superimposed on old endocarditis (*recurrent endocarditis*).

In ulcerative thromboendocarditis, the injury to the valves is very great with formation of massive thrombotic deposits with subsequent necrosis and even formation of pus. The removal of these necrotic areas by the circulating blood leaves an ulcerated surface.

The vegetations of ulcerative thromboendocarditis show the presence of numerous bacteria (mainly cocci) in contradistinction to the few or no organism found in the verrucous thromboendocarditis.

Termination. (*a*) *Complete resolution.*—The vegetations are absorbed and the valves restored to normal state. (*b*) *Ulceration.*—The process may extend, loss of substance of the valves leading to rupture or perforation of the valves; or dislodgement of the vegetations which are carried through the circulation to distant parts, producing embolic phenomena, chiefly in the spleen, kidneys and brain; (*c*) *Organization.*—The vegetations are replaced by fibro-connective tissue leading to (*d*) Adhesion of the valves; (*e*) Contraction and thickening of the valves; (*f*) Calcification of the valves.

Morbid Physiology.—(1) In early stages of the vegetation formation, interference of the proper closing of the valves takes place due to the presence of the nodular excrescences on the valves,—*regurgitation.*

(2) Later stages—the vegetations are organised resulting in *stenosis* of the orifice with or without regurgitation.

(3) Complications—(*a*) In case of simple endocarditis—chance of embolic phenomena—infarction in spleen, kidneys or brain; (*b*) In case of ulcerative endocarditis:—(*i*) Arterial pyæmia, (*ii*) Septic emboli disseminated all over—multiple abscesses in various organs, (*iii*) Rise of temperature—irregular or hectic, (*iv*) Toxæmia. (*c*) In case of stenosis—especially of the mitral valve, stagnation of blood takes place in the left auricle with formation of thrombus which may be dislodged with formation of emboli.

Clinical Classification.—1. *Incompetency of the Valves*—leading to REGURGITATION.

The common causes are:—(*a*) Endocarditis; (*b*) Sclerosis of the valves—(*i*) Arteriosclerotic, (*ii*) Syphilitic; (*c*) Relative insufficiency of the ring (*d*) Rupture of the cusps; (*e*) Congenital malformation, *e.g.*, absence of one cusp or fusion of the cusps.

2. *Fibrosis of the Valves*—leading to STENOSIS.

The common causes are:—(*a*) Endocarditis; (*b*) Sclerosis of the valves; (*c*) Congenital malformation.

Sequence of Events in the Affection of the Valves

Aortic Regurgitation.—(1) Dilatation of the left ventricle as it receives blood from two sources—(*a*) From left auricle, (*b*) Regurgitated from the aorta. (2) Hypertrophy of the left ventricle up to the mark of compensation. (3) When compensation fails, the left ventricle dilates, resulting in relative mitral insufficiency (mitral regurgitation). (4) Dilatation and then hypertrophy of the left auricle up to the mark of compensation ultimately dilatation. (5) Stasis in the pulmonary circulation. (6) Engorgement of the right heart, hypertrophy and then dilatation of right ventricle and then of the right auricle. (7) Systemic congestion. (8) Cardiac liver, œdema. (9) Heart failure, and (10) Death.

Aortic Stenosis.—As it is a gradually developing process the left ventricle gets opportunity to adapt to this abnormal condition. Hence (1) Hypertrophy of the left ventricle, up to the mark of compensation. (2) When compensation fails dilatation of the left ventricle. (3) Subsequent changes same as in aortic regurgitation.

Mitral Regurgitation.—(1) Dilatation of the left auricle as it receives blood from two sources—(*a*) From the pulmonary circulation, and (*b*) Regurgitated blood from the left ventricle. (2) Hypertrophy of the left ventricle and then dilatation. (3) Hypertrophy of the left auricle up to the mark of compensation, failing which, dilatation takes place. (4) Overfilling of the auricle. (5) Subsequent changes similar to aortic regurgitation.

Mitral Stenosis.—(1) Stagnation of blood in the left auricle. (2) Dilatation, hypertrophy and lastly dilatation of the left auricle. (3) Engorgement of the pulmonary circulation. (4) Subsequent changes same as mitral regurgitation.

ATHEROSCLEROSIS

(*Arteriosclerosis*)

Arteries may be divided into two groups—Arteries of the muscular type, and arteries of the elastic type. Atherosclerosis is the affection of the arteries of the elastic type, *e.g.*, the aorta, iliacs, etc.

The nutrition of these vessels is maintained by vasa vasorum derived from the small neighbouring artery, which ramify as capillary network upto the media. In aorta, the capillaries extend upto the outer part of the media but no nutritional capillaries have been found in the inner two-thirds of the media.

It is still an open question how the arterial wall is nourished. It is generally believed that the intima and the inner part of the media are supplied with nutrition from the blood circulating in the lumen of the vessel, while the adventitia and the outer part of the media get their nourishment from the vasa vasorum.

Morbid Anatomy of Atherosclerosis.—This can be divided into four stages:—

(1) In the earliest stage, one observes very small, round, oval or irregular, isolated or diffused, patches of yellowish-white or whitish, slightly elevated streaks or flakes. These flakes are specially found on the dorsal side and often round the mouth of the intercostal or carotid arteries.

(2) At a later stage, some translucent, pale eminences are observed, in addition to the yellowish flakes, which, on section, show a yellow, fatty mass which can be expressed very easily.

(3) In more advanced cases, the plaques become larger and confluent, showing roughening of the surface with actual erosions and ulcerations, allowing a yellow soft fatty mass to be pressed out. These ulcers show areas of deposition of calcareous particles and often fairly large calcified plates, filling up a considerable portion of the aorta, can be seen.

(4) In the most advanced stage, the aorta is converted into a

rigid tube showing ulcers and calcified areas throughout, resulting in great loss of elasticity of the vessel.

Morbid Histology.—The atherosclerotic changes are the result of a continuous process which is best studied histologically.

The earliest stage is characterised by fatty deposits in the so-called Langhans cells, which are branching connective tissue cells present in the superficial subendothelial layer of the intima. These cells are not seen in normal aorta, but appear in large numbers in diseased aorta, and they are specially well seen when they contain fat granules. These cells have been first described by Langhans and later seen by Virchow, and one can see them in very large numbers in any specimen stained with Sudan III or scarlet red.

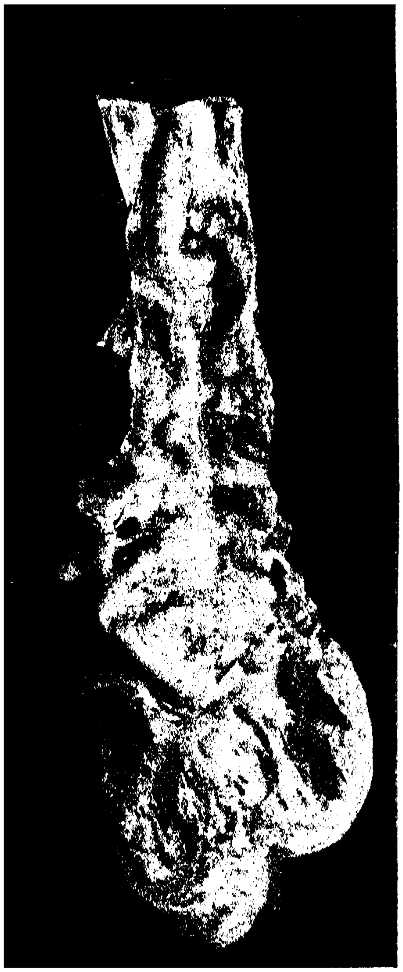


Fig. 319. Atheroma of aorta. (From Path. Mus Carmi. Med. Col.).

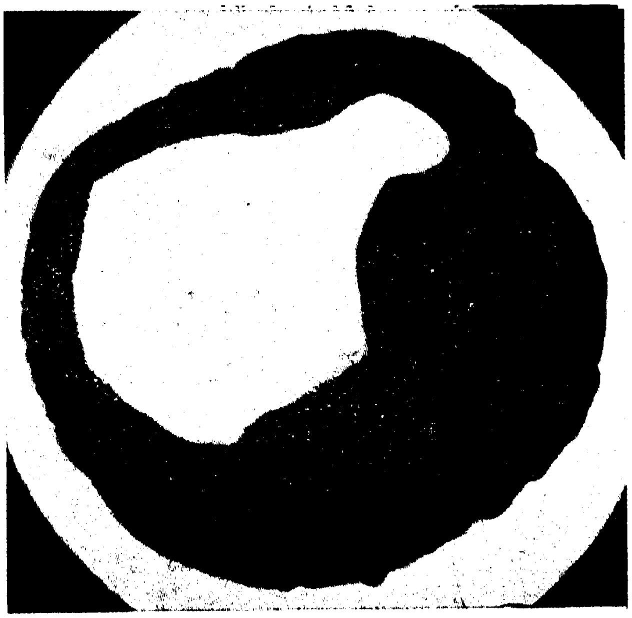


Fig. 320. Atheroma of cerebral vessel showing great thickening and degeneration of the intima.

As the fat deposits are increased, the wandering cells also show fat granules, even the endothelial cells. Ultimately, they are so loaded with fat that they disappear entirely, leaving small spaces filled up with neutral fats, lecithin, cholesterin, cholesterin esters, calcium soaps, etc., forming a microscopic atheromatous focus. The Langhans cells, filled with fat, can be seen at the margin of the focus, which soon die off and are replaced by the fatty mass.

Along with this degenerative process, proliferation of the connective tissue and calcification occur simultaneously, and often with the appearance of new elastic fibrils in the newly formed subendothelial connective tissue.

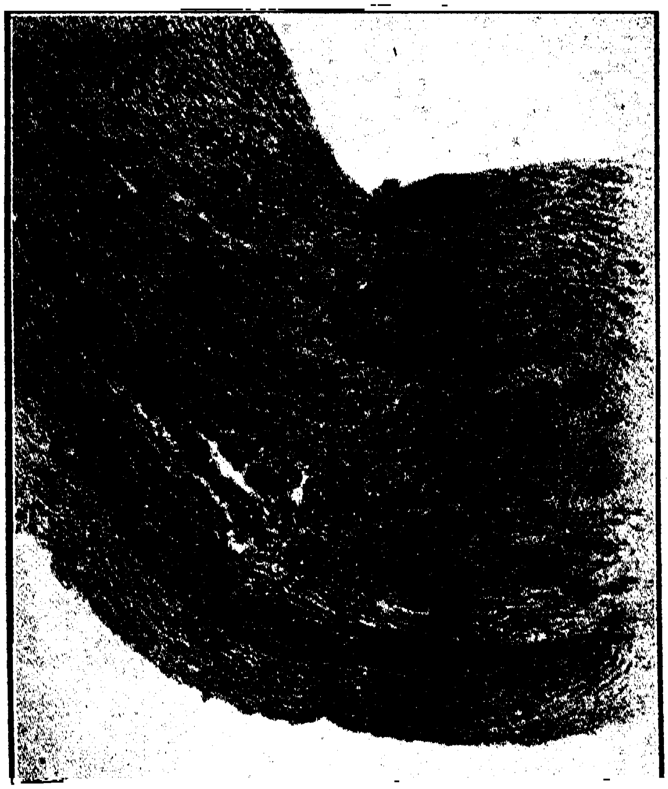


Fig. 321. Portion of **Fig. 320,** Highly Magnified, showing the degenerative changes.

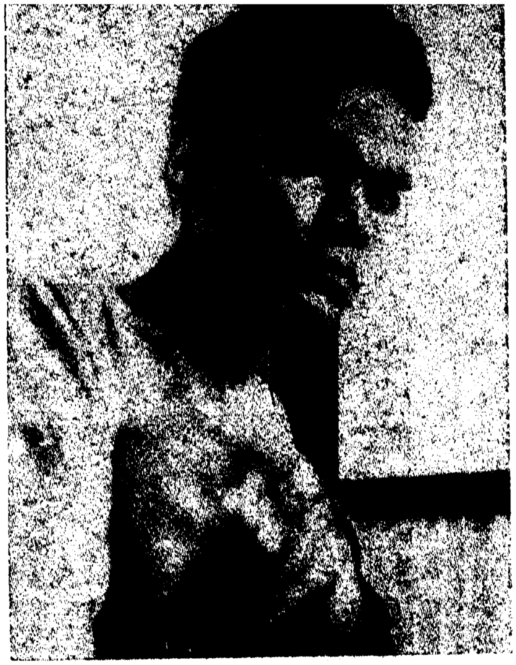


Fig. 322. Aneurism of the aorta.

Thus it is seen that the "fatty changes" or the so-called "atheromatous changes" play the specific rôle and as they bear a definite relationship to the sclerotic process, Marchand is thoroughly justified in using the term "atherosclerosis" for this condition.

The Concept of Atherosclerosis.—Virchow was the first to give accurate histological description of the process. He thought the entire atheromatous process represents "a primary loosening of the intimal layer, due to the infiltration of blood plasma, which is accompanied or followed by a growth of the intimal cells." He also attributed this to be due to irritative process of mechanical origin.'

This original concept was soon forgotten. The unfortunate term "endarteritis deformans," given by Virchow, led the pathologists to view this condition as an inflammatory process, of infectious or toxic origin.

However, modern pathologists believe in Virchow's original concept that this fatty process depends entirely on a deposition of cholesterin ester and is not an inflammatory one.

Experimental Atherosclerosis.—Josué first reported in 1903, that atheromatosis can be produced in rabbits by intravenous injections of adrenalin. Since then innumerable attempts have been made to produce the same in animals.

These attempts have been done in three different lines.—(1) By means of toxic substances, *e.g.*, alcohol, tobacco, adrenalin, etc.; (2) By means of micro-organisms and their toxins, *e.g.*, staphylococci, streptococci, bacillus coli, B. typhosus, B. diphtheria, B. tuberculosis, B. tetani, etc. (3) By feeding with various substances especially cholesterin.

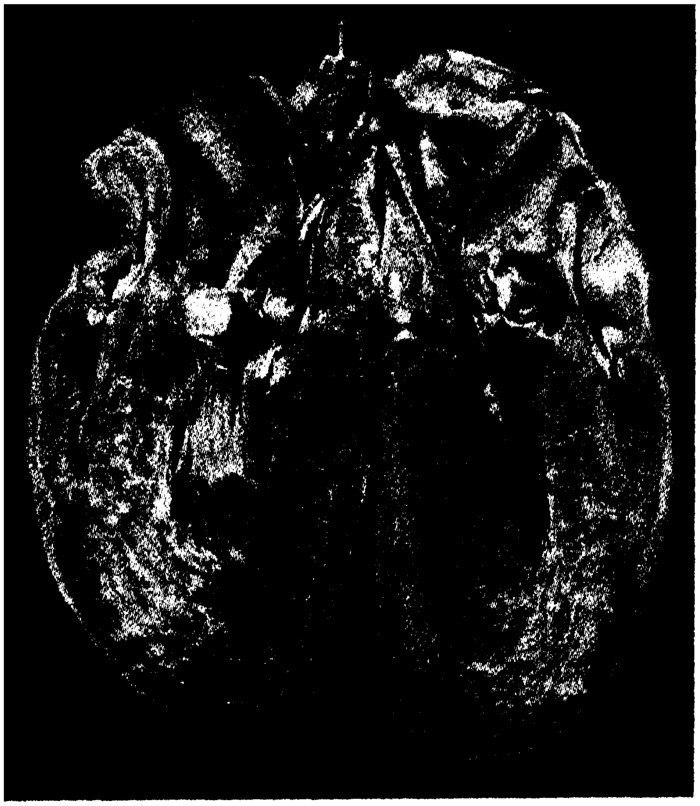


Fig. 323. Fibrosis of myocardium. (From Path. Mus. Carmi. Med. Col.).

By these means, various changes have been observed in the rabbits. Other animals show negative results. These changes are attributed to rise of blood pressure or to contraction of vasa vasorum, followed by necrotic areas in the media, as a result of the ischemia. The toxins of bacteria behaved like adrenalin, but in case of the micro-organisms, inflammatory changes in the vessel wall were invariably found.

A fairly large number of investigators have succeeded to produce, in rabbits, arterial changes resembling closely the early changes of human atherosclerosis. The important difference lies in the presence of hypercholesteræmia in the animals which is not the case in human atherosclerosis.

Pathogenesis.—For the knowledge on pathogenesis of atherosclerosis we are greatly indebted to the German workers Thoma, Marchand, Jores, Benda, Aschoff and many others. The pathogenesis of atherosclerosis is the deposition of fats and lipoids in the cells of the intima, and all the other changes are consequent upon this fatty deposit.

This condition is absolutely non-syphilitic in nature. The term "syphilitic aortitis" is quite clear in itself. As this term implies, syphilis leads to the production of granulation tissue in connection with the vasa vasorum and is characterised by the presence of thickening of the vasa vasorum, with abundance of round cell infiltration and loss of elastic tissue in both the adventitia and media, with no deposition of fat. These changes are quite distinct and can never be mistaken from those of atherosclerosis.

The primary calcification of the media—**the Mœnckeberg type of Arteriosclerosis**—is another distinct type of arteriosclerosis.

SYPHILITIC AORTITIS

As has been mentioned before, syphilis affects all the layers of the wall of the arteries leading to endarteritis, mesarteritis, and periarteritis. This is particularly true in case of small arteries, while in aorta it affects specially the vasa vasorum in the adventitia and consequently the media and then the intima. In the vasa vasorum, again, the perivascular lymphatics are the first sites for the lodgment of the spirochaetes and thus the production of the lesion is characterised by the perivascular infiltration. The media is affected partly by the action of the spirochaetes reaching there by way of the perivascular lymphatics of the vasa vasorum and partly by the obliteration of the vasa vasorum by the endarteritis obliterans resulting in the destruction of the elastic tissue as well as the connective tissue followed by extensive fibrosis. Compensatory increase of connective tissue takes place in the intima.

Morbid Anatomy.—In a specimen of syphilitic aortitis, free from atheromatous changes, the characteristic appearance is the fibrosis or scarring of the inside of the aorta. The scarring appears as wrinkles on the surface generally in the form of longitudinal striations. This appearance is present not only inside the aorta but also on the outside, the arch of the aorta being the common site. The fatty changes along with ulceration and calcification, the characteristic feature of atheroma, are singularly absent. Syphilitic aortitis and atherosclerosis of the aorta, however, are very often associated together.

Histology.—The histological picture is very definite. The earliest changes take place round the vasa vasorum in the adventitia in the form of round celled infiltration—the so called perivascular infiltration. The round cells consist of lymphocytes and plasma cells. The vasa vasorum also shows thickening of the wall, the thickening may extend so far as to complete obliteration of the lumen,—*endarteritis obliterans.* The thickening may be symmetrical and is generally due to the proliferation of the connective tissue cells in the intima. As a sequence also by the direct action of the spirochætes, a considerable amount of to the changes in the adventitia and specially in the vasa vasorum as necrosis and disintegration takes place in the media. The elastic lamellæ and the muscular elements are the chief sufferers, with formation of breaches in numerous places. The destruction of the elastic tissue in the media forms the main change in syphilitic aortitis, and when widespread, naturally, leads to dilatation of the wall with formation of aneurism. The necrosis of the elastic and muscle tissue are generally replaced by inelastic fibrous tissue. The intima, although does not suffer directly from any action of the spirochætes, yet it shows gross change in the form of great thickening. This is entirely compensatory. The intima at the mouth of the coronary artery may be involved leading to obstruction of these vessels and may lead to sudden death.

ANEURISM

An aneurism is a localised dilatation of an artery. Although an aneurism may occur in any artery, the aorta and its main branches are the usual sites.

Etiology.—Aneurism is generally formed due to the weakness of the vessel wall with or without the presence of increased blood pressure. The weakness of the wall is generally caused by the necrosis of the media *e.g.,* in syphilitic aortitis, suppuration extending to the media, by a septic embolus or extension from a neighbouring abscess or from a tuberculous focus. The involvement of the media is essential to the formation of an aneurism. There may be congenital weakness of the wall of the vessel.

Varieties.—*True aneurism*—in which the outer coat of the vessel form the sac. *False aneurism* (*traumatic aneurism*)—in which the surrounding tissue forms the wall of the sac *e.g.,* after rupture of the vessel into the perivascular tissue. *Dissecting aneurism*—in which the blood passes between the different layers of the wall of the vessel after splitting it up. It may rupture externally or even internally again into its own lumen. *Fusiform aneurism*—when the portion of the vessel (generally the aorta) is uniformly dilated to some distance. *Saccular aneurism*—when the dilatation is more localised so that the aneurism appears as a pouch and may communicate with the vessel lumen by a narrow opening. *Circoid aneurism*—when a mass of anastomosing and dilated vessels are grouped together in the form of a single mass. It is really not a true aneurism. *Arterio-venous aneurism*—when there is an abnormal communication between an artery and a vein. The vein dilates to a considerable extent and pulsates along with the artery.

Other Arterial Changes.—These take place in different ways according to the functional needs of the different parts of the vascular system during the different phases of human life. (1) *Senile Atrophy* is characterised by the disappearance of the elastic and muscular layers with replacement by fibrous tissue; (2) *Senile Calcification* of the vessels takes place mainly in the vessels of the muscular type with deposition of considerable amount of calcareous particles in the media; (3) *Hyaline Changes of the Small Arteries.*—These generally take place in the vessels supplying the organs, with deposition of a hyaline material in the subendothelial layer, leading to narrowing or obliteration of the vessel lumen.

CHAPTER XXXV

MENINGITIS

Etiology.—*Primary.*—Meningococcic (cerebro-spinal fever).

Secondary.—1. Tuberculous—secondary to tuberculosis of gland, etc. 2. Pneumococcic—secondary to pneumonia, pericarditis, etc. 3. Pyogenic—secondary to affections of the cranium, otitis media or terminal infection in chronic diseases. 4. Other infectious diseases, *e.g.,* syphilis, typhoid, influenza, diphtheria, gonorrhœa. etc. 5. Constitutional affections, *e.g.*, nephritis, cachexia, etc.

Varieties. 1. Pachymeningitis—inflammation of the dura mater. 2. Leptomeningitis—inflammation of the pia and the arachnoid. *According to the situation.*—

1. Vertical or cortical when affecting the meninges at the vertex of the brain. 2. Basal—when affecting the meninges at the base of the brain. 3. Spinal—when affecting the spinal meninges. 4. Temporal—when affecting membranes over the temporal lobe of the brain. 5. Posterior basic—generally affects children under one year.

Morbid Anatomy.—There is intense congestion of the pia-arachnoid. The exudation first appears as slight accumulation of lymph between the pia-arachnoid subsequently changing into fibrinous, sero-fibrinous and ultimately fibrino-purulent forms. The exudation is most marked at the base of the brain with great thickening of the meninges. The exudation may also appear over the vertex and is found mainly along the larger sulci and fissures. In extreme cases the whole of the vertex may be covered with thick fibrino-purulent exudate.

The spinal meninges are also affected chiefly on the posterior surface and more on the dorso-lumbar region than over the cervical region. There is increased amount of cerebro-spinal fluid in the meningeal space as well as inside the ventricles which are dilated. The fluid is generally turbid except in cases of tuberculous and syphilitic affections. The inflammation often extends to the subjacent brain substance which may show evidence of hyperæmia and even hæmorrhages and may be quite soft (encephalitis). In chronic conditions the meninges are greatly thickened and may be adherent to the surrounding structures.

Histology.—Identical to the changes seen in pericarditis.

Morbid Physiology.—Fever, leucocytosis, rigidity of muscles, retraction of neck, Kernig's sign, headache, delirium, tremor—due to irritation of the meninges and brain by toxæmia, increased intracranial pressure and direct inflammation.

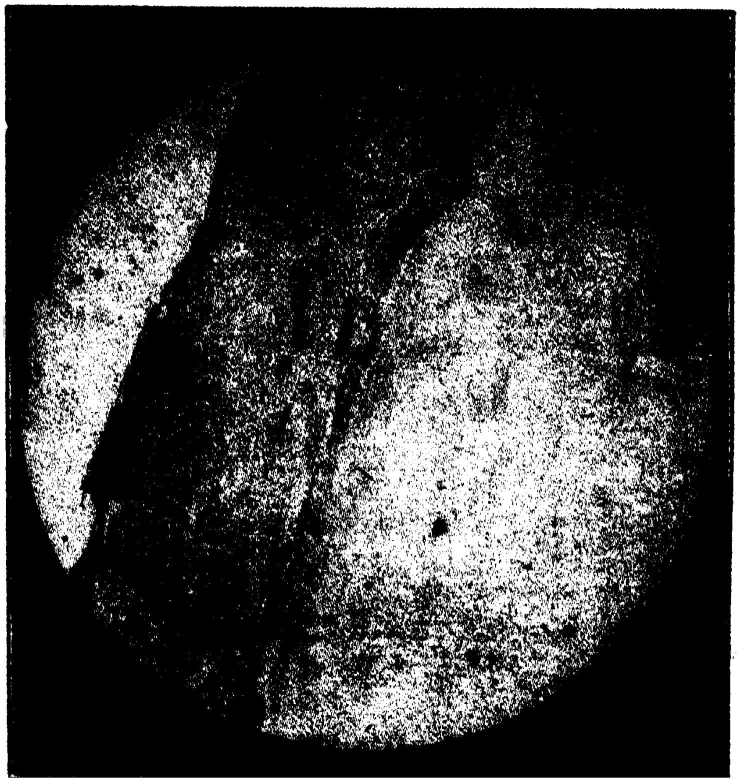


Fig. 324. Acute meningitis, showing great thickening of the meninges.

Brudzinski's sign—due to the irritation of the respective muscles by the same factors.

Orthotonos position is generally due to the fact that in this position maximum amount of space is available for the accumulation of the fluid in the meningeal space.

Changes in the cerebro-spinal fluid (*see* "Examination of C. S. Fluid").

CHAPTER XXXVI

DISEASES OF THE RESPIRATORY SYSTEM

ANATOMICAL AND PHYSIOLOGICAL CONSIDERATION OF THE LUNGS

The terminal bronchiole breaks up into one, two or more *respiratory bronchioles,* which, again, divides into 2 to 11 *alveolar ducts,* from which arise the *alveolar sacs* and *alveoli.* The connecting portion between the alveolar sacs and the alveolar ducts is called the *atrium.*

The *pulmonary unit* consists of all the above structures from the respiratory bronchioles to the alveoli in addition to the blood and lymph vessels, connective tissue, nerves etc. Each of the structures composing the pulmonary unit may now be considered. *Respiratory bronchiole* starts with a diameter of about 0.5 mm. and goes down to 0.24 mm. and is lined by ciliated columnar epithelium containing no goblet cells. There is no cartilage in the wall. This is so called as it takes part in the process of respiration. *Alveolar ducts* are very thin walled tubes measuring about 0.19 mm. from which blind polyhedral pouches originate which constitute the *alveolar sacs* and the *alveoli* with the space between the alveolar duct and the alveolar sac forming what is known as the atrium. Some authors do not regard the existance of any such anatomical structure as the atrium. There are septa between the alveoli which form the wall of the alveolar ducts. The septum is beset with a large number of branching net work of wavy reticular fibres and is very rich in capillaries, which anastomose freely. The reticular fibres are more abundant than the elastic fibres. Collagen fibrils surround the mouths of the alveolar sacs.

The cells lining the alveoli.—Our knowledge at the present moment regarding these cells is very imperfect. It is not yet settled whether these cells are epithelial or mesenchymal. In the fœtal lung, the alveolar cells are more cubical, becoming flattened with the onset of inspiration. In adult lung this flattened respiratory epithelium comes into intimate relationship with the surrounding capillaries. Apart from these cells which are generally termed as the "septal cells," there exists a fair number of free macrophages or alveolar phagocytes and are variously called "the dust cells," "heart failure cells." It has been said that these cells take their origin from the septal cells.

The lymphatics of the lung belong to two main sets, one in the pleura, and the other in the pulmonary tissue, both draining into the lymph nodes at the hilum of the lung. These two sets communicate with each other very infrequently because of the presence of a very large number of valves in the pleural set which control the flow of the lymph in not allowing it to pass to the pulmonary tissue. The pulmonary lymphatics divide into several groups including those of the bronchi, of the pulmonary artery and of the pulmonary vein. There are no lymphatic vessels beyond the alveolar ducts. The lymphoid tissue increases considerably as age advances owing to the irritation of the dust and coal particles which are being constantly deposited in the lymphatics by the endothelial phagocytes.

ACUTE LOBAR PNEUMONIA.

(*Also called fibrinous or croupous pneumonia.*)

Etiology.—The pneumococcus of Frænkel is the commonest cause. It may also be due to bacillus mucosus capsulatus, and a few other organisms. Friedlander's bacillus produces a glary or sticky exudate, with generally a bad prognosis. (*For the different types of Pneumococcus and the mortality caused by each see page* 109.).

Morbid anatomy.—Acute lobar pneumonia as the term implies affects the whole lobe. According to the distribution of the lesion at the commencement it is termed—*Apical.*—when the apex of the lung is affected. *Massive.*—when considerable portion of one or both the lungs are affected. *Central pneumonia.*—when the inflammation is deep-seated and placed centrally in a lobe. *Double pneumonia.*—when both the lungs are affected. *Migratory or creeping pneumonia.*—when one lobe after another is successively involved.

In all cases of lobar pneumonia the lung is found to be larger and more bulky than normal; it is heavier, solid in consistency due to the consolidation assuming a liver-like consistency hence called *hepatisation,* the free margins are rounded off, indentations of ribs are present on the surface. It cuts with ease and if a small portion of the lung is put on water it sinks immediately. On scraping the cut surface little plugs of fibrin comes out. The lung is either red, reddish-grey in colour according to the stage of the disease. The pleura is nearly always involved which becomes more or less rough due to the presence of the deposition of fibrin.

Stages of Pneumonia.—(*a*) THE STAGE OF ACUTE CONGESTION. This stage is characterised by distension of the capillaries, exudation of the serum, desquamation of the alveolar cells, and hæmorrhage into the alveoli.

(*b*) THE STAGE OF RED HEPATISATION.—*Naked eye appearance.*—The lobe of the lung becomes airless and the consolidated area is bright-red in colour, very friable, and stands above the normal tissue. *Microscopically.*—The pulmonary capillaries are engorged and the air cells are filled with very large number of red blood cells entangled by an abundance of fine network of fibrin. Polymorphonuclear leucocytes appear in large number and also phagocytic mononuclear cells and desquamated alveolar cells. The fibrinous exudation is not only seen inside the alveoli but also on the adjacent pleura (fibrinous pleurisy) and even in the small bronchi (fibrinous bronchitis). Large number of pneumococci may be seen either free or inside the leucocytes and other phagocytic cells.

(*c*) THE STAGE OF GREY HEPATISATION.—*Naked eye appearance.*—The lung is greyish in colour and the tissue is very friable. The change of colour is due to disappearance of red blood cells. *Microscopically.*—(*i*) Contents of the air-spaces retracted from the wall. (*ii*) Red blood cells are absorbed, polymorphs are in great excess and are in various stages of disintegration; (*iii*) Congestion of the vessels of the inter-alveolar wall is less marked. (*iv*) A section, stained with Weigert's fibrin stain, shows the fibrin filaments most beautifully entangling the leucocytes. One sees the fibrin filaments passing in the form of bands through openings in the wall of the alveolus, thus connecting one with the other.

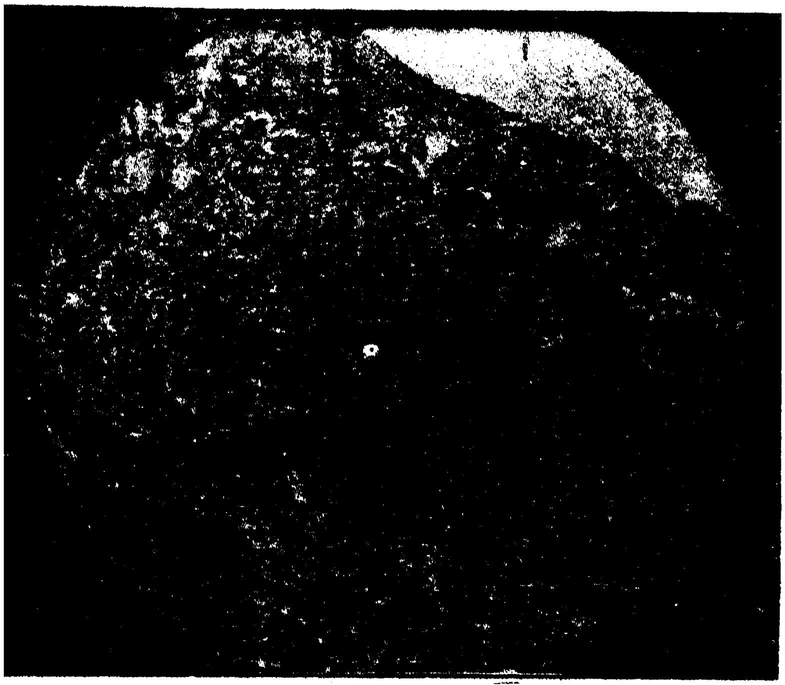


Fig. 325. Acute lobar pneumonia. (Low power). The air spaces are full of fibrinous network with numerous red cells and leucocytes

(*d*) THE STAGE OF RESOLUTION.—In favourable circumstances absorption of the inflammatory products takes place, after they have been liquefied by means of autolytic ferments produced by the leucocytes and some of the contents may be expectorated out. The alveolar cells are regenerated and the alveolus gradually becomes normal. No sharp line can be drawn between the stages of grey hepatization and resolution.

In unfavourable circumstances the resolution may be incomplete, the fibrinous exudation remains unabsorbed and ultimately becomes organized (fibrosis, induration or carnification). The organization of the fibrin generally starts at the points of contact while passing through the openings in the alveolar walls. Secondary infection or various complications may occur.

Complications.—(*i*) Localised abscess formation or diffuse suppurative condition. (*ii*) Gangrene of the lung. (*iii*) Empyema due to the softening and involvement of the pleura. (*iv*) Adhesion of the layers of the pleura. (*v*) Chronic interstitial pneumonia—fibroid phthisis. (*vi*) Inflammation of other serous sacs, *e.g.*; pericarditis, endocarditis, meningitis, peritonitis, arthritis, etc.

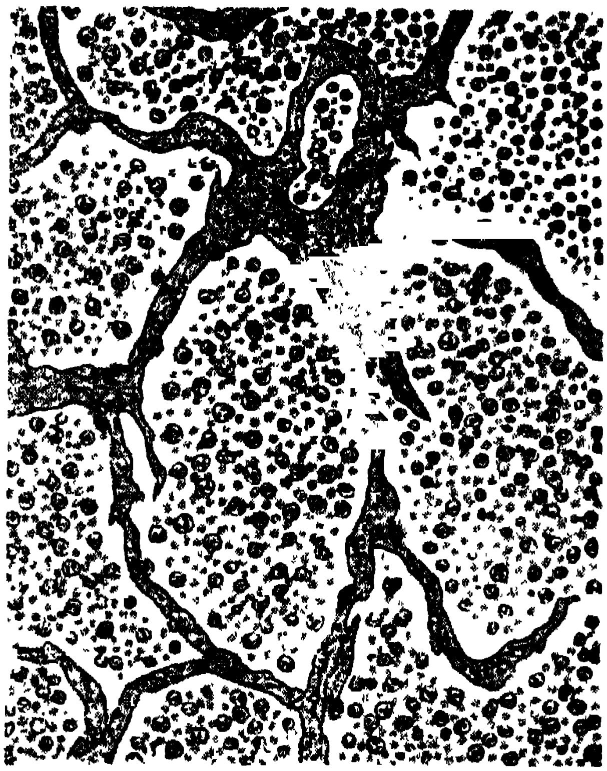


Fig. 326. Acute pneumonia, (Grey hepatisation), showing leucocytes and red cells in the air-spaces of the lung.

Morbid Physiology.—Pneumonia is a form of septicæmia with intense toxæmia. That it is septicæmia is explained by the presence of the associated inflammation of other serous membranes. The toxæmia is responsible for all the symptoms of the disease—fever, dyspnœa, rapid pulse, etc. The difficulty of breathing is mainly due to toxæmia and not to the diminution of the oxygenating surface or respiratory obstruction. This is explained by the fact that the area of the affection of the lung remains exactly the same before and after the crisis but all the symptoms disappear with the onset of crisis which is nothing but due to the neutralisation of the toxin in the system. On the other hand, a very small area of consolidation *e.g.*, in central pneumonia may be associated with great difficulty of breathing. Death depends on the intensity of toxæmia and not on the area of the lung involved. *Pain* is mainly due to the associated pleurisy. It is the stretching of the pleural adhesions which produce pain rather than the rubbing of the inflamed pleural surfaces against each other.

Rusty sputum depends on the presence of the red blood cells in the sputum which is very viscid on account of the presence of a large amount of fibrin in it.

he *crepitant rales* are caused either by the air bubbling through the viscid exudation or due to sudden separation of the walls of the alveoli held together by the sticky exudate.

BRONCHOPNEUMONIA

Syn.--Bronchopneumonia.--Because the infection extends from the bronchioles to the alveoli. *Lobular pneumonia* or focal pneumonia.—Because of the distribution of the lesions according to the lobules which never affects the whole lobe. *Catarrhal pneumonia.*—Because the lesion is of catarrhal type of inflammation.

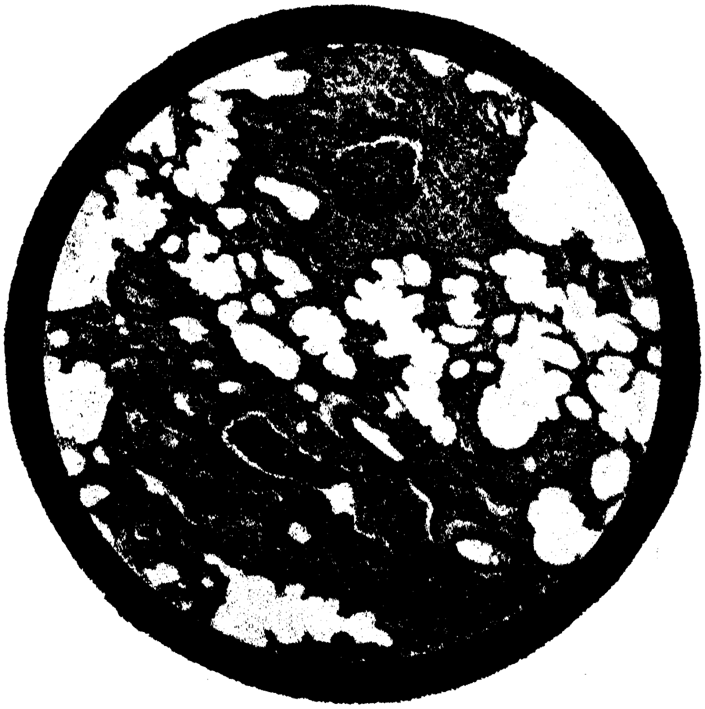


Fig. 327. Acute lobular pneumonia (bronchopneumonia). The lumen of the bronchioles and air-spaces are filled with inflammatory exudates forming the consolidation.

Etiology.—This generally occurs in the old and very young persons. 1. *Primary.* --Infection with pneumococcus, pneumobacillus, strepto and staphylococci, B. influenza, B. tuberculosis, B. pertussis, B. pestis, B. dipththeria, glanders, actinomyces, etc. 2. *Secondary.*—(*a*) As a complication of measles, whooping cough, smallpox, typhoid fever, erysepelas, etc. (*b*) Aspiration pneumonia.

Morbid anatomy.—The lungs present reddish or purplish consolidated patches (lobules) with intervening normal spongy lung tissue. These patches may be very minute, consisting only of a few alveoli or they may be of any grade up to large areas over a lobe. Small pieces of the lung sink in water whereas large pieces may float. The pleural surface usually remains glossy but may show a thin fibrinous exudate, if any focus of consolidation lie just under the surface.

Morbid histology.—The microscopical picture is quite variable. The inflammation starts in the smaller interlobular branches of the bronchi. The inflammation spreads to the walls of the neighbouring air cavities. The affected bronchioles are blocked with leucocytes and serous or fibrinous exudate and the portions of the lung tissue supplied by them undergo collapse. The epithelial lining of the bronchioles may remain intact but desquamation may also take place. The inflammatory process spreads along its interior to the colapsed area, which becomes the site of congestion and filled up with the emigrated leucocytes. Acute distension of some of the neighbouring air cavities around a bronchopneumonic focus takes place with subsequent collapse. Intervening lung tissue shows intense congestion and œdema. Exudation of fibrin is most inconspicuous while in acute lobar pneumonia it is most prominent and characteristic. Acute inflammation of pleura takes place in the areas in the immediate contact with the affected portions of the lung.

Termination.—1. Resolution and restoration of normal lung. 2. Persistence of the collapse of the affected area of the lung. 3. Chronic interstitial pneumonia resulting in great thickening of the interlobar septa and formation of bronchiectatic cavities. 4. Suppuration or gangrene of the portion of the lung.

Hypostatic pneumonia.—(*a*) Hypostatic congestion and œdema of the dependent parts of the lungs lead to this condition when this pass to the stage of consolidation. (*b*) A subacute form of inflammation of the terminal bronchioles and air vesicles—the cavities become filled up with catarrhal cells, fibrin and leucocytes.

Interstitial pneumonia.—A subacute or chronic condition, characterised by overgrowth of connective tissue framework of the lung, resulting from continued irritation.

Classification—(*a*) Pneumonokoniosis—resulting from inhalation of dust and other particles. (*b*) Following acute or subacute inflammatory conditions of the lung or pleura, *e.g.*, imperfectly resolved lobar or lobular pneumonia, collapse, irritation by the foreign body, pleurisy, etc. (*c*) Due to the action of bacterial and other poisons, *e.g.*, tuberculosis, syphilis, leprosy, influenza, etc.

Other forms of Pneumonia.

Terminal Pneumonia.—This takes place in cases who have been in a comatose condition for some time before death, *e.g.*, in cerebral hæmorrhage.

Postoperative Pneumonia.—This takes place by aspiration of septic material into the bronchus during general anæsthesia, particularly in operations on the month and tonsils. This is also called *aspiration pneumonia.*

Chemical Pneumonia.—This takes place by inhalation of irritating and poisonous gases as used during the World War.

TUBERCULOSIS OF THE LUNG

[For the general consideration of Tuberculosis, *see* page 406.]

The lungs are the most frequent seat of tuberculosis; the infection takes place most commonly through the air and is generally caused by the human type of the tubercle bacillus. The process may be acute or chronic according to the virulence of the organism and the resistance offered by the tissues. The lesion necessarily shows itself in a multiplicity of forms.

In a previous chapter we have discussed how infection with tuberculosis takes place, how it spreads throughout the body, the nature of the primary and the secondâry infection and also the Koch's phenomenon. The understanding of the immuno-biological processes leading to the development of tuberculosis is essential. The concept of *allergy*, the most important factor in tuberculosis, depends mainly on the understanding of ,these processes and these, again, on the "phenomenon of Koch."

As mentioned before, Koch observed that subcutaneous injection of tubercle bacillus in a guinea-pig behaves quite differently in immunized and non-immunized animals. He observed that, when tubercle bacillus is injected into a normal guinea-pig, nothing happens at the site of inoculation for about 2 weeks, when a hard nodule appears which ulcerates and remains so until the death of the animal. But quite a different picture is seen when similar inoculation is done to a guinea-pig which had been infected with tuberculosis 4 to 6 weeks previously. In this animal, the lesion appears at the site of inoculation as early as the second or third day, and the skin becomes necrotic and ulcerated within a few days; but healing of this ulcer takes place very rapidly and often completely, without even involving the neighbouring glands. This phenomenon, known as the *Koch phenomenon,* is the basis for the conception of the allergy.

By *allergy* we mean a condition of altered susceptibility or hypersensitiveness of the tissue and their associated cells. This period, or the anaphylactic period is characterised by an inflammatory exudate with abundance of leucocytes round the focus. This exudation mechanically stops further passage of tubercule bacillus in the lymph channels; the leucocytes also destroy large number of them which attempt to penetrate through the exudate. The Koch phenomenon also shows that this anaphylactic period is followed by an increasing resistance, resulting in the productive form of pulmonary tuberculosis.

Tuberculosis of the lung thus shows itself in three stages. The primary lesion, secondary (anaphylactic) exudative stage and finally of increased resistance, the productive stage.

Types of Reaction in Pulmonary Tuberculosis.—Anatomically five different types are met with. These are:—

1. *Productive phthisis.*—In this type the formation of specific granulation tissue is the most prominent feature. Fibrosis, hyalinization, and collapse of the alveolar sacs by indurative process are the characteristic findings. Healing process (organisation) advances rapidly. This represents the evidence of immunity of the tissues. The epitheloid cells, giant cells, round celled infiltration and deposition of fibrous tissue are the prominent histological findings in this type of lesion. The epitheloid cell, characterised by a large cell with feebly stained clear cytoplasm and a large vesicular nucleus, is the chief type of cells in this form of reaction. These cells are highly phagocytic and are derived from the histiocytes, either tissue histiocytes of the septal cells or blood histiocytes, endothelial leucocytes or monocytes. In other conditions, these cells are variously known as dust cells, heart failure cells, foam cells, etc.

2. *Exudative Phthisis.*—In this type the formation of exudative pneumonia is the most prominent feature. This is generally seen in the form of acute miliary tuberculosis or pneumonic or bronchopneumonic phthisis. Exudation of fibrin and cellular elements, *e.g.*, large endothelial cells with practically no giant cells are the characteristic findings. This represents allergic phenomena of the tissues and generally occurs in instances where the infective dose is large with poorly developed immunity. The defence force is thus early overpowered, resulting in an acute, rapidly spreading lesions as no reaction comes from the tissue to localise it. The histological

evidence of this absence of defence reaction is the complete absence of reticulum between the affected cells. This constitutes what is termed by the clinician "galloping consumption" or "phthisis florida." The exudative reaction is thus a sign of lowered resistance and indicates rapidly progressive destruction of the tissue.

3. *Productive Exudative Phthisis.*—(Fibro-caseous Tuberculosis) This is the form which is most commonly met with and is characterised by presence of the healing process (fibrosis) on the one hand, necrosis (caseation and cavity-formation) on the other. Caseation is generally caused by two factors: (1.) the toxin of the tubercle bacillus, and (2) the avascularity of the tissue. The thickening of the pleura, interlobular septa, the walls of the bronchi and the blood vessels are also present. Of the blood vessels, not only the adventitia but also the intima show proliferative changes with production of endarteritis obliterans leading to great narrowing, even obliteration of the lumen of the vessels. It is often found that a fairly big sized blood vessel traverses a cavity with no support from the surrounding structure. The endarteritic changes obliterating the lumen serve a very useful purpose in these cases, as without that fatal hæmorrhage might occur at any moment. The area thus shows proliferative and productive zone of fibroblastic connective tissue, associated with similar changes of elastic and reticulum fibres. The tissue surrounding this area usually shows both serous and cellular exudative changes. The cellular elements, *e.g.*, epitheloid cells, giant cells, round cells, etc., are also characteristic.

4. *Mixed Phthisis.*—The anatomical, specially, the histological picture is altered by the presence of secondary infection with consequent complications.

5. Acute miliary tuberculosis.—See page 405.

PNEUMOTHORAX

The condition known as *pneumothorax* is produced by the entrance of air or gas into the pleural cavity. This is nearly always accompanied by serous or purulent effusion. It is then called *hydropneumothorax* or *pyopneumothorax* respectively.

Etiology.—A rupture of a tuberculous cavity into the pleura is the commonest cause. Other lesions which may lead to the production of a communication of the pleural cavity with the exterior are the rupture of an abscess, bronchiectasis or a septic infarct, or even emphysematous bullæ. A fractured rib or a punctured wound from

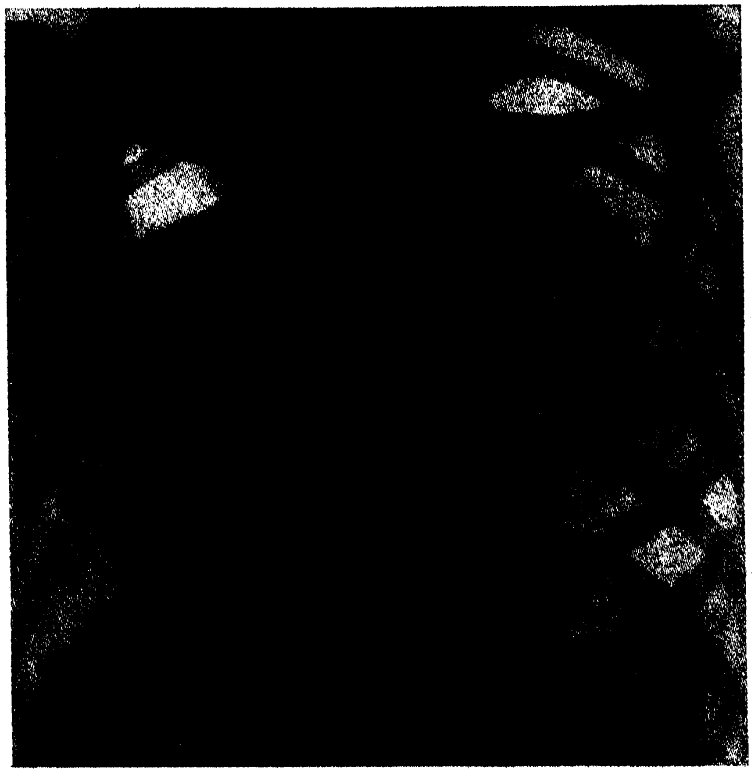


Fig. 328. Radiograph of the chest showing tuberculous broncho-pneumonic areas. A cavity is seen on the right subclavicular region.

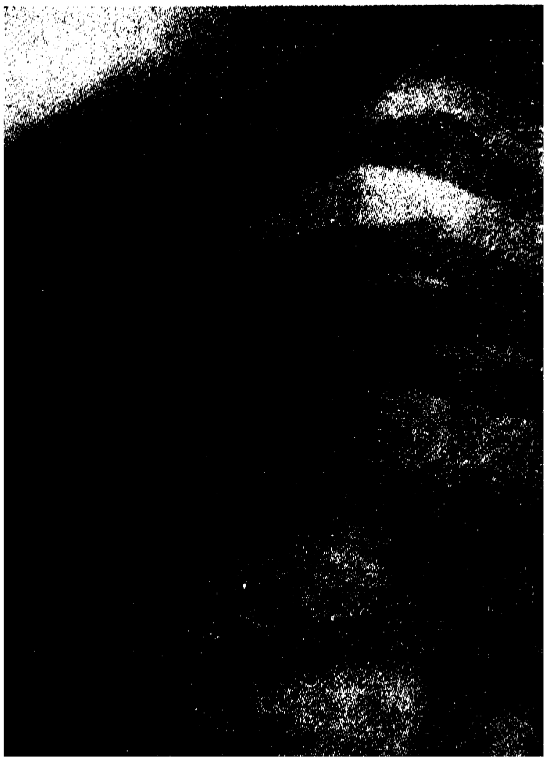


Fig. 329. Radiograph of the chest showing annular shadow of cavity in the subclavicular region.

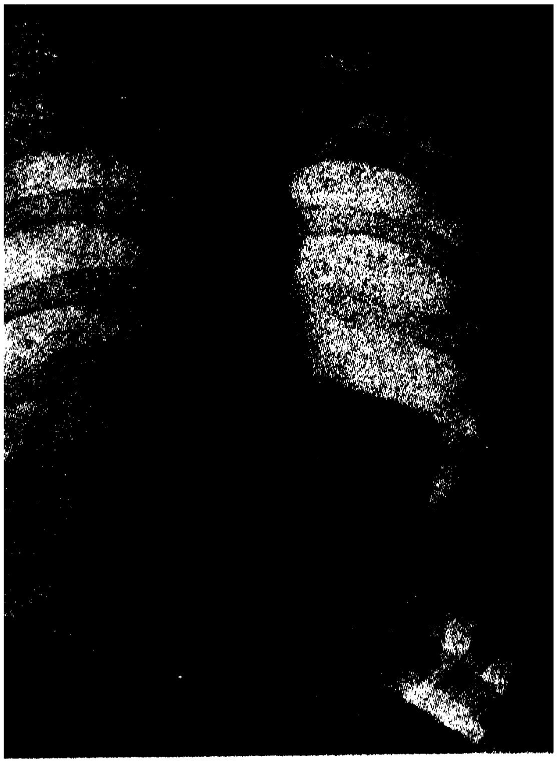


Fig. 330. Radiograph of the chest showing collapse of the left lung by artificial pneumothorax.

exterior, rupture of gastric ulcer, perforation of the œsophagus may also produce pneumothorax, or if associated with sepsis, as they usually are, pyopneumothorax. There may also be spontaneous or idiopathic pneumothorax. In artificial pneumothorax air or gas is introduced therapeutically in order to give rest to the tuberculous lung.

Morbid Anatomy.—The lung is collapsed unless adhesions are present. The heart and the mediastinum are displaced to the opposite side and the diaphragm pushed downwards on the affected side.

These conditions cannot be seen in a museum specimen if the necropsy is done by the ordinary methods. The writer has performed several necropsies according to the method of Koch by which the specimen can be preserved in the museum showing at the same time the topographical relationship between the pneumothorax cavity and the other structures very clearly. The displacement of the heart and the mediastinum, also the diaphragm and the effect of adhesions, if present, on the collapse of the lung are very well shown on the accompanying figures.

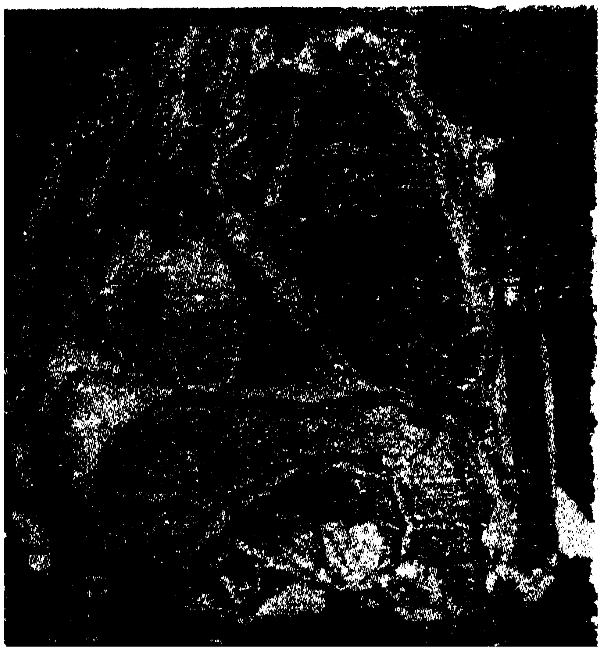


Fig. 331. Pyopnemothorax opening into the abdominal cavity through the diaphragm. The opening is seen at the base of the left chest. Note displacement of the heart and the mediastinum. A band of adhesion at the apex prevents complete collapse of the lung. (From Path. Mus. Carmi. Med. Col.).

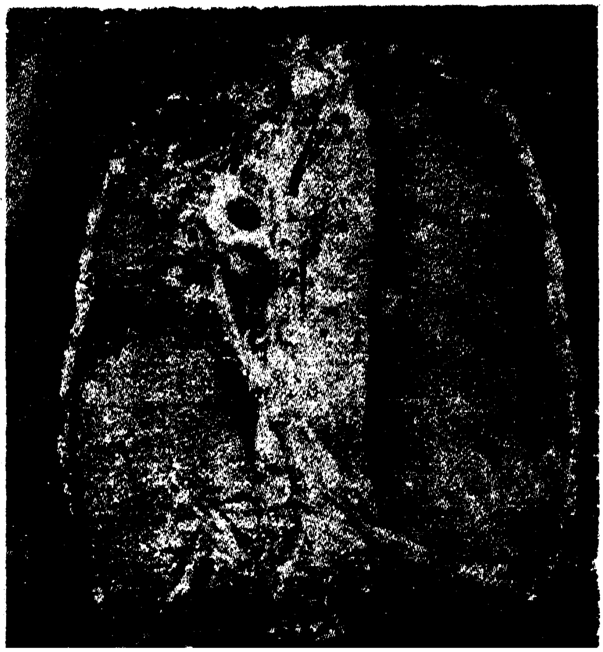


Fig. 332. Pyopneumothorax. Note the great downward displacement of the left dome of the diaphragm (From Path. Mus. Carmi. Med. Col.).

CHAPTER XXXVII

DISEASES OF THE KIDNEY

The kidney is essentially an excretory organ, and has a three-fold function: (1) To eliminate the end-products of protein metabolism—urea, uric acid, creatinin, etc.; (2) To maintain the normal composition and volume of blood; (3) To regulate the neutrality reaction of the tissue. This is maintained by the excretion of most of the inorganic salts of both acid (sulphates, phosphates, and chlorides) and basic radicles (Na, K, Ca, Mg, etc.).

Histology.—The functionating elements of the kidney consist of a large number of units which have been termed "nephrons" by Braus. The nephron consists of one glomerulus and the corresponding tubule commencing from the Malpighian body and ending into the collecting tubule. The Malpighian body consists of the glomerulus and the glomerular capsule. The glomerulus again consists of the network of capillaries lined by endothelium but showing no definite cell outline. The glomerular capsule or Bowman's Capsule consists of a double layer of epithelium, one (the visceral layer) lining the glomerulus and the other (the parietal) which rests on a homogeneous basement membrane. The lumen between these two layers is continued into the neck of the tubule. The tubule is subdivided into several portions, *e.g.*, the neck, proximal convoluted tubule, descending loop of Henley, distal convoluted tubule which terminates into the collecting tubule. The epithelium lining the different portions of the nephron differs in type The cells lining the proximal convoluted tubule have several distinctive characters. They are of low columnar type with a round nucleus and are of granular appearance. The cytoplasm stains more readily with eosin than the other segments of the tubules found in the cortex. The descending loop of Henley is lined by thin and flattened epithelium with large oval nuclei. The cells lining the ascending limb being more cuboidal. The cells of the distal convoluted tubules are of low columnar type.

The interstitial tissue forms a minor factor in the histology. The older pathologists used to give great importance to the interstitial tissue but now we know that any increase in the growth of the interstitial tissue is only to fill us the gap left by any loss of the nephron.

Blood Supply of the Nephron.—The glomerulus receives a twig (the afferent vessel) from the interlobular artery, which divides into a capillary tuft which, in its turn, combines to form one vessel (the efferent vessel) which leaves the glomerulus and divides again into numerous branches which supply the arterial blood to the other part of the nephron—the tubules. Thus it is seen that the nutrient supply of the tubule must pass through the glomerulus and so any obstruction in the glomerulus at once leads to degeneration of the tubules.

Physiology.—Many investigations have been done to determine the function of the nephron. Bowman was the first to find the afferent and the efferent arteries of the glomerulus and believed that the function of the glomerulus is to separate water from the blood which dissolves the solids (urea, etc.) secreted by the tubules. Normally salts, and, in pathological conditions, sugar, albumen and foreign substances are also passed by the glomerulus. Ludwig regarded the elimination of water through the glomerulus as due to purely physical forces, the Bowman's capsule acting as a simple filter through which water and all the constituents of urine are filtered, excepting only the colloids. Heidenhein tried to put these theories on experimental basis and came to the conclusion that the whole process is of secretory in character, by means of vital activity of the epithelium of the capsule and the tubule. The glomerulus secretes water and salts and the cells of the convoluted tubules and of the ascending loop of Henley secrete the specific constituents of the urine, *e.g.*, urea, uric acid, pigments, etc.

This supposes that the renal cell has got the power of discrimination of a very high order. It not only detects the presence of a foreigner in the blood which is expelled immediately, but also acts as a chemist in possession of a very well equipped laboratory in detecting any quantitative change in the composition of normal constituents. Thus the renal cell takes no notice of the presence of sugar if it is present in the blood in the proportion to 0.1 per cent.; but as soon it rises to 0.2-0.3 per cent, it is at once activated and secretion of sugar follows immediately.

None of these theories has received full acceptance by the physiologists. Both from the point of view of development as well as from histological evidence, the kidney cannot be regarded as a secretory gland. Kidney is mesodermal in origin, whereas the typical secretory glands are either outgrowths from the gut and hence endodermal, or, from the surface of the body and so epidermal in origin.

The modern theory is based on fishing out what is best in these theories. In 1917 Cushny suggested that it is possible to harmonise the apparent contradictions in the two theories by assuming that a protein-free fluid containing all the diffusible substances of the plasma is filtered from the blood in the glomerulus. During its passage through the tubules water and certain substances useful for the organism (the threshhold substances) are reabsorbed and at the same time leads to the concentration of the substances (non-threshhold substances) in the urine. Cushny accepts that filtration of all the constituents of the blood excepting the colloids occurs in the glomerulus by a purely physical force, the blood pressure of the glomerular capillaries supplying the forces. The filtrate passes through the tubules where a considerable quantity of water and certain solid constituents are absorbed by the tubular epithelium by an active vital process and not by a process of passive diffusion. Resorption of these substances takes place from the convoluted tubules that are required to maintain the normal threshhold level in the blood, *e.g.*, NaCl. Little or no resorption takes place of those substances which are not needed to maintain such thereshold level, nor of substances which are foreign to the body. The loop of Henley acts as a pressure reservoir leading to the stasis of the filtrate in the tubules, favouring the absorption. There is no definite evidence that the cells of the convoluted tubules actually secrete anything into the lumen.

Thus we find that almost all the constituents of urine are passed through the glomerulus which is much thinner than the urine as it comes out. The normal consistency and specific gravity of urine are maintained by resorption of some elements from the glomerular filtrate.

The fluid re-absorbed by the tubules was at first assumed to be of the same composition as that of Locke's solution. Meyers found that it is not so simple: some amount of urea and possibly some other substances are also re-absorbed.

Various experiments have been made with a view to proving the above processes. Wearn and Richards in 1924 were able to introduce a fine capillary into the Bowman's space of the kidney of the frog. By this means they could isolate the glomerular filtrate which, when analysed, was found to be protein-free but contained chloride and glucose, whereas the bladder-urine did not show the presence of these things at the same time. This proves definitely that reabsorption takes place in the tubule. White and Schmidt have confirmed these results and found that reabsorption of sugar and chlorides have taken place in proximal convoluted tubule. Other substances found in the glomerular fluid of frogs are urea, potassium and foreign substances such as dyes and iron salts when injected.

Apart from these two well-established theories as regards the physiology of urine formation, a third factor has also attracted the attention of numerous workers, *e.g.*, whether there is also a specific secretion of the tubules. Heidenhein believed this to be a prominent factor. Since then various other workers studied this by several methods of which the process of vital staining has shown, that when a dye is directly introduced into the capsular fluid, the convoluted tubules show the same appearance as that found histologically when the dye is introduced through the systemic circulation, thus disproving the theory.

As regards the secretion of abnormal constituents of the urine it has been found that albumen is chiefly eliminated by the glomerulus. Starr induced albuminuria experimentally by vasoconstrictor agents, *e.g.*, CO_2 inhalation, adrenalin, etc, which may be thought to lead to asphyxia and so increased permeability of the glomerulus. Toxins may also lead to the same thing.

CLASSIFICATION OF BRIGHT'S DISEASE

One hundred years have just passed by, since Richard Bright (1827) described the kidney diseases which have since been termed by his name.

Ever since the time of Bright, or even much earlier, the kidney diseases have attracted the attention of the scientists, and it is also fairly long since the relationship of kidney diseases with the excretion of water, salt, nitrogen and their relation with œdema, rise of blood pressure, hypertrophy of the heart, uræmia, etc., have been established; but it was in 1914 when Volhard and Fahr followed by a host of observers and in 1917 when Cushny established his " modern theory " that the knowledge on these affections have been directed in the right direction, There is a great want for a proper classification of Bright's disease and a great confusion exists in the literature at the present moment.

Objection to the Present Classification.—The use of the terms "large white kidney," "small white kidney," "red granular kidney," "small granular kidney," "small contracted kidney," etc., are, strictly speaking, lay-man's terms. If the kidney is small and it appears granular on the surface, we call that "small granular kidney," ignoring altogether that this may take place in chronic inflammatory changes of the glomerulus or more commonly in arteriolosclerosis of the kidney with no trace of any inflammation, the whole process being in the vessels, *i.e.*, extrarenal and also differing widely in clinical manifestations. This comes to be the same thing as is meant what MacCullum quotes for comparison "small blue lung," or, "large red liver."

The clinicians and some pathologists, again, classify very often as "parenchymatous" or "interstitial" nephritis. These terms are strictly speaking histological terms and by parenchymatous inflammation, the main localization of the lesion is meant to be in the tubules which structures, really, play very little active part in the process.

Nephritis may be classified on different standpoints but none of them are free from objections. They may be on—

(1) *Etiological Basis.*—But, (*a*) Same etiological factor (streptococcus) causes various other lesions. (*b*) Same lesions are caused by different factors. (*c*) Etiology is unknown in many instances.

(2) *Clinical Basis.*—But, (*a*) Different lesions may produce identical clinical features. (*b*) Clinical picture varies widely in different stages of the same lesion. The initial stage differs widely from the terminal stage clinically. Moreover, one can rarely follow the whole course of the affection from the begining to the end. We generally see the terminal stage and rarely the initial and the intermediate ones. (*c*) General vascular changes or cardiac affections are often responsible for the production of renal manifestations and so the lesion is really extrarenal and not renal. (*d*) Meyer's classification based on the "modern theory" is good. He classifies nephritis into two groups: (*i*) Hydræmic type.—This depends on primary injury to the glomerulus which leads to increased permeability and hence loss of serum protein, thus the total colloid osmotic pressure of the plasma is decreased. Œdema results from this disturbance of the

normal relationship between the capillary blood pressure and colloid osmotic pressure. (*ii*) Azotemic type.—The damage of the tubular epithelium leads to reabsorption of some urea along with the chlorides. This is responsible for the rise of the percentage of blood urea.

(3) *Pathological Basis.*—This is the best one but the difficulty of the pathologist is also great. He sees only the end results of the human cases on the post mortem table.

Thus it is essential, the classification of nephritis must rest on the observations of both the clinician and the pathologist, and that not on a particular phase of the disease, but observed over a fairly long period during life and also after death. This is impossible either by the clinician or the pathologist alone. It is essential to correlate the clinical, biochemical, and pathological findings in all the different stages of the affection in order to come to a definite classification.

Russell classifies Bright's disease under two distinct pathological processes, one of them is due to arterial degeneration: an ischæmic nephritis (arterio-sclerotic kidney). The other is an inflammatory reaction to bacterial or other toxin: a toxic nephritis. Ischæmic nephritis may occur independently of toxic nephritis, but chronic toxic nephritis is generally complicated by cardiovascular hypertrophy and thus by secondary ischæmic nephritis. She further classifies toxic nephritis into two main kinds. In one the parenchymatous degeneration is the main change with little or no inflammatory reaction. This corresponds to the nephrosis of Volhard and Fahr. The other kind of toxic nephritis is characterised by high grade of inflammatory reaction.

It is said that if all the kidney specimens of all the museums of the world are collected in one place, they can be grouped under the following heads:—

(1) Glomerulonephritis
(2) Arteriosclerotic kidney } 90 per cent. of all medical affections of the kidney.
(3) Acute interstitial nephritis and suppurative Nephritis.

(4) Nephrosis.—By this is meant the degenerative conditions limited entirely to the tubules and without any inflammatory changes. This generally includes (*a*) Lipoid nephrosis or fatty kidney, (*b*) Amyloid nephrosis, (*c*) Bichloride kidney or nephrosis after bichloride of mercury poisoning, (*d*) Kidney of pregnancy, (*e*) Cholera kidney, (*f*) Epstein's nephrosis.

Thus we see that the etiological factor of one form of nephrosis is quite different from the other. Clinically, again, they have very little in common. Pathologically, it is found, that some form of nephrosis, *e.g.*, lipoid nephrosis may restore itself to normal structure and even may functionate normally but in other form, *e.g.*, amyloid nephrosis restoration is impossible.

INFLAMMATORY CONDITIONS OF THE KIDNEY

From the above it is seen that the inflammatory conditions of the kidney are found in the following forms.—

1. Glomerulonephritis.
2. Acute interstitial nephritis without suppurations.
3. Suppurative nephritis including pyelitis, perinephric abscess and pyonephrosis.

GLOMERULONEPHRITIS

Etiology.—Infections with bacteria themselves or the toxin liberated by them are the chief agents. Sudden exposure to cold is also a very important contributory factor.

The toxin is eliminated through the kidneys and acts on the various elements of the nephron. In streptococcus the main lesion is caused in the glomerulus whereas in diphtheria or scarlet fever we find the interstitial tissue greatly inflamed, and the condition is known as acute interstitial nephri-

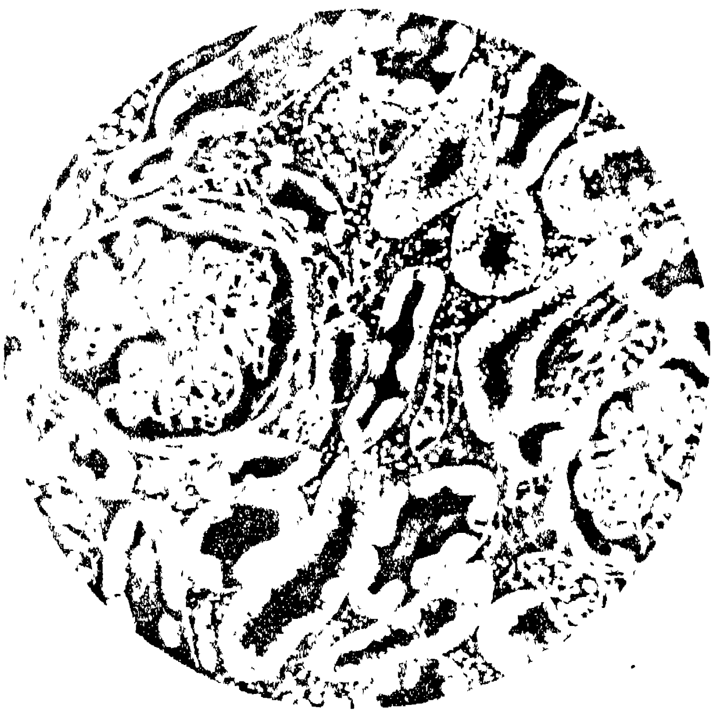


Fig. 333. Acute glomerulonephritis, (high power) showing—(*i*) Engorgement of both the glomerular and intertubular capillaries, (*ii*) Cloudy swelling and desquamation of the epithelium of the glomerulus, (*iii*) Round cell infiltration of the intertubular tissue.

tis. It is very peculiar in that there is absence of polymorpho-nuclear leucocytes in the lesions; it consists of lymphocytes and plasma cells. In other toxins, for example in cholera the main action is on the tubule and practically speaking the glomerulus is free. Different toxins act on different parts producing different conditions and different pathological changes.

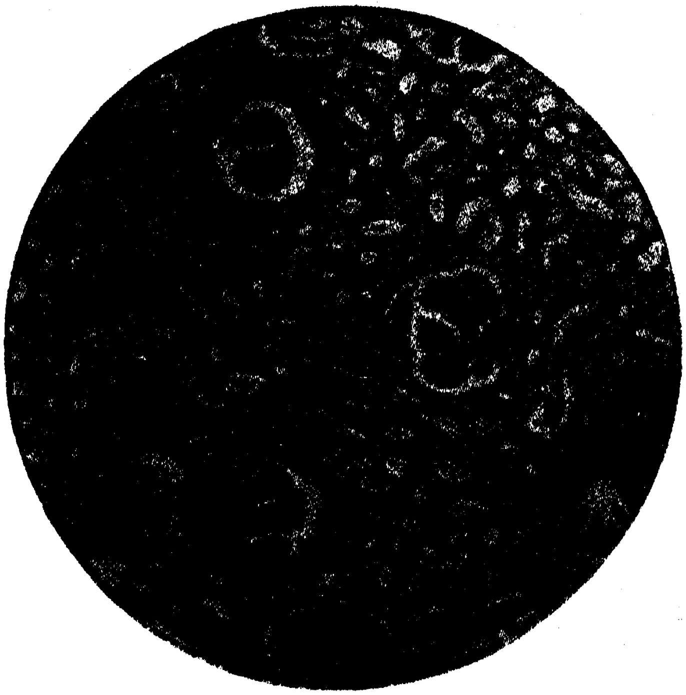


Fig. 334. Subacute glomerulonephritis showing great thickening of the capsular epithelium forming the appearance of a crescent—the so-called epithelial crescent.

The streptococcus generally from the throat and tonsils infects the kidney in two ways: directly by their presence in the glomerulus or by the toxin alone. Thus we find the lesion mainly lie in the glomeruli, and as such from the histological and functional standpoints we have the glomerulus as the agent which suffers most, because it has got to bear the brunt of the attack.

Varieties of Glomerulonephritis.—(A) *Focal*—(*i*) Embolic glomerulonephritis.—This generally occurs in the course of subacute streptococcal endocarditis. This is *the classical example* of focal nephritis. (*ii*) Non-embolic focal glomerulonephritis—also called focal hæmorrhagic glomerulonephritis. This is *the commonest form* of nephritis.

(*B*) *Diffuse*—(*i*) Acute diffuse glomerulonephritis. Volhard found low grade streptococcal infection of the tonsil in 125 cases out of 179. (*ii*) Subacute diffuse glomerulonephritis, or the chronic parenchymatous nephritis of old writers. (*iii*) Chronic diffuse glomerulonephritis, or the secondary contracted kidney with various adjectives of the old writers.

In case of embolic glomerulonephritis, generally, we find a focus, as such the function is not greatly involved. In the case of diffuse glomerulonephritis we find a good deal of glomeruli involved, thus we find a gross damage to the kidney structure and the whole metabolism. In case of glomerulonephritis in the acute state from the pathological point of view we find very few specimens as when the patient is dead we find the end product of the lesion, but, this can be produced experimentally. In this case we generally find a very large amount of damage to the capillary loops. In the majority of instances, if it is diffuse, we find a very large number of capillaries lost. In early stages we find a good deal of expansion in the capsular space. So in early stages acute hæmorrhagic nephritis or hæmorrhage is the most common and peculiar thing.

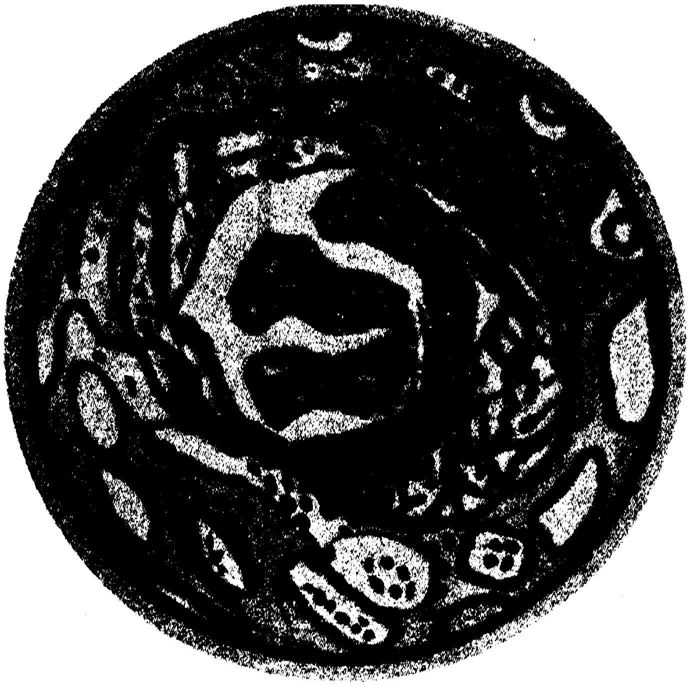


Fig. 335. Subacute glomerulonephritis. One glomerulus of Fig. 334 greatly magnified to show the epithelial crescent.

The lesion of acute glomerulo-nephritis which is generally caused by streptococcus leads to the production of swelling and dilatation of the capillary loops, and on account of the thrombosis, in course of time, we find total absence of red corpuscles in it. No blood can pass through these glomeruli. We know the efferent vessels of the glomeruli before they come to the formation of veins supply the tubules. That means, in glomerular lesion leading to thrombosis, it will lead to vascular disturbance of the tubules, and as such the tubules will suffer on account of loss of function of glomeruli, and, secondly, on account of vascular interference due to the occlusion of the glomeruli. This will lead to actual damage to the tubules. In course of time increase of cellular elements in the glomeruli takes place. That is due to the inflammatory exudates or proliferation of the epithelial cells. The essential process due to these epithelial changes is the cessation of blood flow through the glomeruli and as such the glomeruli are absolutely out of function. The increase of cellular elements also leads to pressure on these capillary tufts leading to damage. So, sooner or later, there is hyalinization of these glomeruli.

In case of sub-acute and sub-chronic glomerulonephritis the process is more or less the same. Here also we find the three fundamental changes—the formation of epithelial crescents, increase of cellular elements inside the capsules, and subsequently hyalinization of glomeruli, and this leads to degeneration of the tubules. The epithelial crescents are formed by the proliferation of the capsular epithelium which assumes the appearance of a crescentic mass. These epithelial cresents are characteristic changes of the glomerulus in the subacute stage of glomerulonephritis.

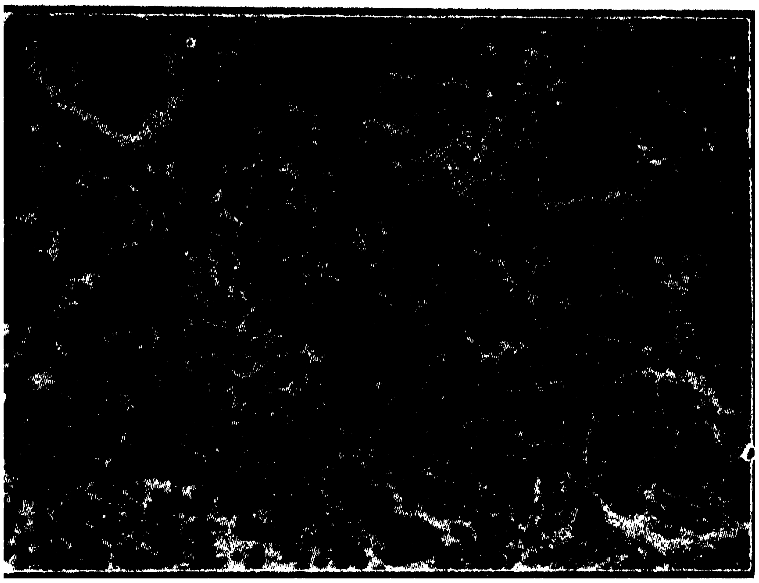


Fig. 336. Acute interstitial nephritis in diphtheria, showing great proliferation of cells in the interstitial tissue.

In case of chronic glomerulonephritis we find practically speaking the same thing further pronounced, and at the same time on account of the loss of a large number of glomeruli and tubules there is a good quantity of renal substances lost, and this place will be filled up by the proliferation of interstitial tissue. There is no inflammatory condition of the interstitial tissue itself. Subsequently we also find that the vessels are thickened, and so in very chronic conditions it is very difficult to differentiate as to whether it is chronic diffuse glomerulonephritis or arterio-sclerotic kidney.

As regards the clinical picture and pathological changes due to these things, in acute glomerulonephritis we generally find that a definite period generally elapses between the onset of the infection and the occurrence of the nephritis. We do not generally get the lesion immediately with the onset of endocarditis, generally some interval is passed.

In case of acute glomerulonephritis there is generally œdema and also rise of blood pressure, and in severe cases there is deficiency of kidney function. In this condition the blood picture gives the finding that the total uric acid and creatine, etc., are increased. This increase indicates the seriousness of the disease. The total non-protein nitrogen goes up to 80 or occasionally to 100 milligrammes or even more. In this condition hæmorrhage or hæmaturia is a very common finding.

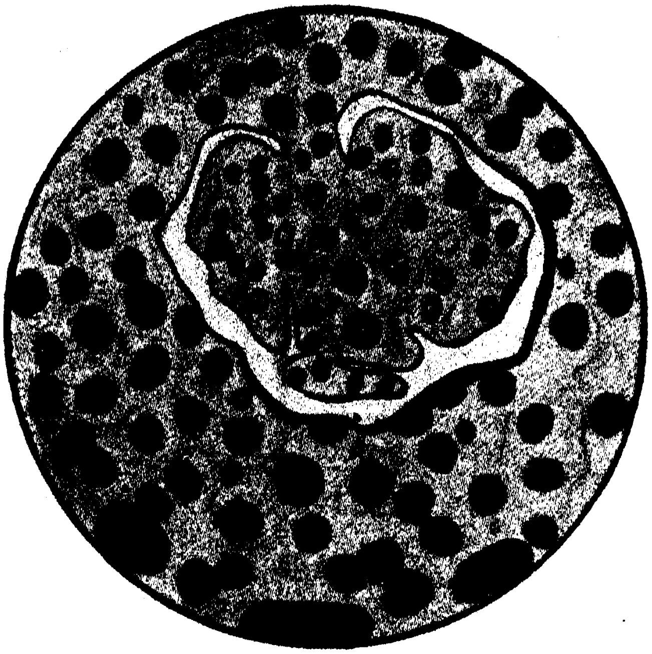


Fig. 337. Acute interstitial nephritis in diphtheria, accumulation of round cells and plasma cells only with no polymorphonuclear leucocytes.

In case of sub-acute and chronic glomerulonephritis the symptoms are pronounced and in some cases the renal deficiency may be absolute.

Chronic glomerulonephritis generally produces three different periods. First, apparently the patient seems to have recovered from the sub-acute or acute condition, but after the acute symptom is passed off the patient still shows symptoms of slight albuminuria indicating that the recovery is not complete. There are repeated attacks of the same streptococcus which was the causative agent of the original attack. With repeated attacks the blood pressure increases and it is maintained at a high level. In the second period, persistent hyper-tension and loss of kidney reserve are the most important features. There is also presence of slight albuminuria, casts, etc. In the third period the renal insufficiency is absolute, and it generally terminates in six months to one year. In case of chronic glomerulonephritis it may lead to very chronic uræmia.

Morbid Physiology.—SEQUENCE OF EVENTS IN OBSTRUCTION TO THE PASSAGE OF BLOOD THROUGH THE GLOMERULUS; *e.g.*, IN ACUTE DIFFUSE GLOMERULONEPHRITIS.—(1) Obstruction to the flow leads to diminution of glomerular filtrate which leads to diminished flow of urine which may even lead to complete suppression. (2) Subsequent accumulation of those substances in the blood which are usually excreted through the glomerulus, *e.g.*, water and nitrogenous waste products. (3) Increase of blood pressure. This takes place due to two factors: (*a*) Increase in the fluid volume of the blood, (*b*) Widespread constriction of peripheral and splanchnic arterioles.

The affections of the tubules add three important clinical manifestations to those of the glomerular lesion:—

(1) Increase in albuminuria, the result of the affection of the epithelium of the capsule which forms the blind end of the tubule.

(2) Presence of tubular cells and casts in the urine, the result of destruction and desquamation of the lining epithelium of the convoluted tubules.

(3) Diminution in the specific gravity of urine the result of diminution in the reabsorptive function of the tubules and hence less concentration of the urine.

Correlation between the Kidney Pathology and Clinical Picture.

Main Pathological Changes.—	*Main Clinical Manifestations.*—
1. Gradual hyalinization and occlusion of large number of glomeruli.	1. Persistant and gradual increase in blood pressure.
2. Loss of function and nutritional disturbances of the tubules leading to their disappearance and replacement by proliferation of interstitial tissue.	2. Gradual increase of the renal insufficiency until it becomes absolute and manifested by inability to concentrate urine and accumulation of waste products in blood specially the increase in the nitrogeneous products, *e.g.*, non-protein nitrogen urea, creatinin, etc.
3. Thickening of the walls of the afferent arteries of the glomeruli.	3. Uræmia and death.

ARTERIOSCLEROTIC KIDNEY

Varieties of Arteriosclerotic Kidney.—*Focal*—In this form the kidney shows one or more irregular scars on the surface, due to localised loss of nephron followed by deposition of fibrous tissue.

Diffuse.—In this form, a very large number of the arterioles is simultaneously affected, with extensive destruction of the glomeruli and subsequently of the tubules. This gives a granular appearance

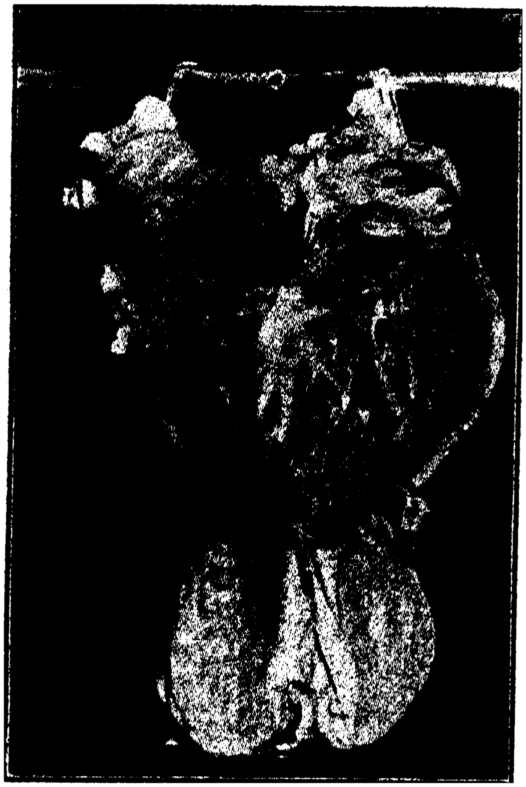


Fig. 338. Arteriosclerotic kidney, atheroma of the aorta and hypertrophy of the heart. (Path. Mus. Carmi. Med. Col).

to the whole kidney. On account of the lesion being diffuse throughout the organ, and as the arterioles are mainly affected, it is better to use the term arteri*ol*osclerosis of the kidney.

(*a*) The size is generally smaller than normal; (*b*) The surface is rough and shows numerous granules and may present one or more cysts of varying sizes; (*c*) On section, it cuts with difficulty—(*i*) The cortex is much atrophied, often reduced to a line in thickness; (*ii*) The medulla may also be atrophied; (*iii*) The blood vessels show greatly thickened walls with great diminution of the lumen; (*d*) The capsule is much thickened and firmly adherent to the surface of the kidney, so that it can be stripped off with much difficulty, and that only with much laceration of the subjacent tissue.

Morbid Histology.— The histological appearance differs to a great extent, accord-

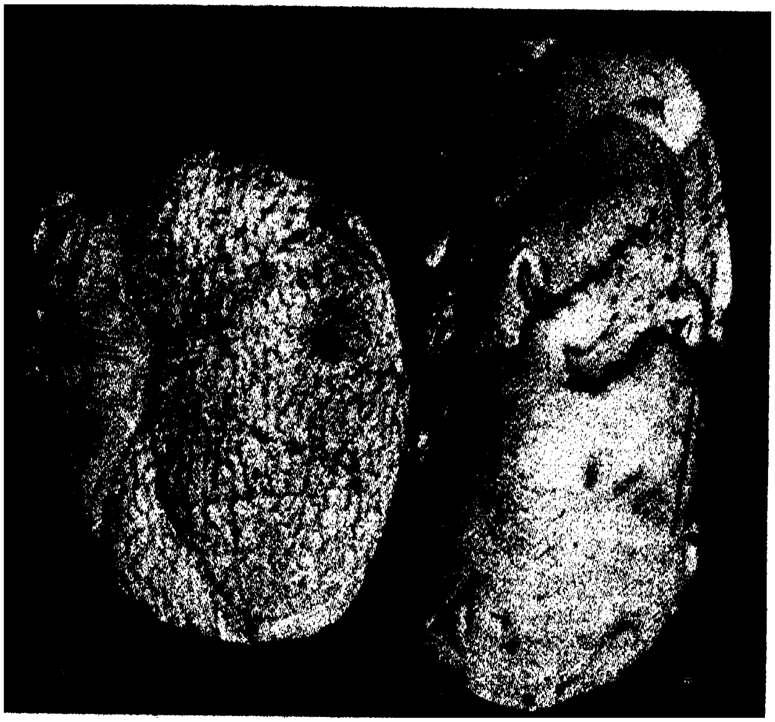


Fig. 339. Two varieties of arteriosclerotic kidney. (From Path. Mus. Carmi. Med. Col.).

ing to the variety of the affection. In the arteriosclerotic kidney, the blood vessels show widespread affection throughout the organ, leading to a considerable interference with the blood supply, leading to atrophic and degenerative changes of both the glomeruli and the tubules. The vessels and a large number of the glomeruli are obliterated, with consequent atrophy and degeneration of the tubules. The loss of the nephron is followed by deposition of fibrous tissue, the contraction of which leads to irregularity on the surface of the organ.

Morbid Physiology.—This is generally associated with general arteriosclerosis, hypertrophy of the heart, and high blood pressure.

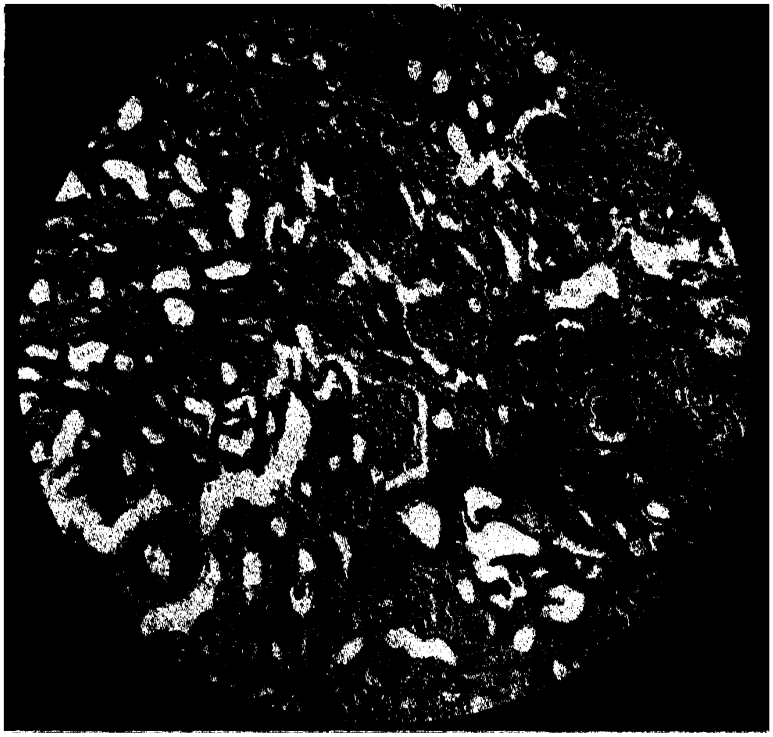


Fig. 340. Arteriosclerotic kidney.
Showing.—
(*i*) Progressive overgrowth of the interstitial tissue causing separation of the tubules which show
(*ii*) Atrophic changes in the epithelium.
(*iii*) Obliteration of the glomerules.
(*iv*) Great thickening of the blood vessels.

CHOLERA KIDNEY

The toxin of the cholera vibrio produces a profound change in the kidneys—so much so that the most dreadful and common complication depends mainly on the renal disturbances with the production of uræmia, which when once developed is very difficult, often impossible, to control.

Inspite of grave changes in the renal function, it is a curious fact that naked eye or even microscopic appearances are so slight that they are quite out of proportion to the manifestations produced by these changes. Even in cases dead of uræmia, the changes are not always constant. Naked eye and microscopical appearances sometimes fail to reveal any gross change beyond some congestion of the organs. Some of the cases show degenerative changes in the epithelium of the convoluted tubules. Extensive hæmorrhages are observed in the tissues of the organ, specially in the cortex, both in the glomeruli and the intertubular tissues. In the cases dead of uræmia Rogers found that in perfusing fluid through the organ, post-mortem, more pressure was required in the cases of cholera kidneys than in those from other diseases.

Affection of the Kidney by Cholera Toxin.—Fuji studied experimentally after injection of a cholera bacillus emulsion in animals and came to the following conclusions: " There is no theory that can satisfactorily explain the anuria in cholera. Theories are based on post-mortem observations without supplementing the facts by experimental pathology. In my experiments the cholera toxin early brings forth nephrosis of the urinary canaliculi, and then causes glomerular nephritis. The granular degeneration of the tubuli contorti is seen very early and reaches its height 8 to 24 hours after infection, when the interior of the canaliculi becomes almost obliterated. The kidney gradually becomes anæmic and finally bloodless. Turbidity and swelling then decreases by degrees. The cellular protoplasm turns into a granular substance (which is well stained with eosin) and serous substance. They stay in the shape of drops in the tubuli contorti. Later on they are discharged into the tube while the height of the cell decreases and the urinary canal becomes wide. Then the blood in the kidney either becomes normal again or remains in a state of congestion or hæmorrhage."

Cholera generally attacks the young persons with no previous damage of the kidneys but it is not uncommon to find people with previous damages of the kidneys suffering from cholera subsequently, and these are the cases which generally run into uræmic complications often with fatal results. In these cases the kidneys may show gross mainfestations either of the previous changes of the renal tissue alone or with those of cholera in addition.

CHAPTER XXXVIII

DISEASES OF THE ALIMENTARY TRACT

PEPTIC ULCERS

These are ulcers produced by the proteolytic action of the gastric secretion upon the devitalised mucous membrane, and usually associated with hypersecretion of acid. The common factors responsible for lowering the vitality of the gastric wall are hæmorrhage, embolism, thrombosis, spasm and infection of the gastric vessels. In animal, ulcers resembling human peptic ulcers can be produced by injection of streptococcus. It is believed by some that localised infection of the mucous membrane by streptococcus is responsible for peptic ulcer in man.

Pathogenesis of gastro-duodenal ulcer is a mystery. It is a noticeable feature that in spite of the active function of the gastric juice to digest all animal tissues the normal gastric mucosa itself has got a remarkable immunity against this action, but this immunity falls through immediately when the mucous membrane is damaged by any reason.

It is also a common experience, specially in experimental animals to find digestion of the mucous membrane taking place very rapidly after introduction of corrosive poisons. Even violent vomiting or other mechanical causes which produce minute haemorrhagic points in the mucosa, show ulcerated points, due to the digestion of the devitalized wall, although the size may be so small as that of a pin head or even smaller. Thus we find that the gastric secretion although it cannot digest the normal mucous membrane of the stomach will do so with remarkable rapidity as soon as it is devitalized. This is one function, the other one is more surprising and is rather paradoxical. This is the remarkable capacity of the gastric mucosa to heal completely and rapidly however extensive the damage or the resulting digestion might be. In fact, MacCullum reports that " I have removed large squares of the mucosa of the dog's stomach, only to find, a short time later, such complete healing that it was impossible to say where the ulcer had been."

In cases of the peptic ulcer of the stomach, we find, on the contrary, in spite of efforts at healing, a tendency to persist for a very long time and to progress deeper and deeper into the wall of the stomach and even to perforation.

The minds of the pathologists have been puzzled by this peculiarity of experimental ulcer in the stomach from the gastric ulcer as seen in the human being. It has also been noticed that in the human stomach smaller ulcers are very often met with due to various factors and are being called erosions.

Various workers, notably Aschoff, regard the pathogenesis of ulcer formation under two constant phases, *e.g.,* (*i*) the development of erosions and (*ii*) the development of ulcer.

The apparent contradictory opinions as regards the pathogenesis of gastroduodenal ulcer is mainly explained by the fact that the two constant phases of the problem are not definitely separated. The opinions which are apparently contradictory belong to two groups, "one is valid for the formation of the erosion, while the other point of view is valid for the genesis of the ulcer." (Aschoff). These two phases require separation from the fact that in experimental animals it had never been possible to produce defects which could be kept persisting for a fairly long time so as to become a real chronic ulcer. These have healed up completely within a short time.

Thus from the behaviour of these different conditions we must group them under two heads:

1. Mucosal erosions *i.e.,* a fresh loss of mucosal tissue due to any factor. This acute loss generally extends to the mucosa and the superficial layers of submucosa with no involvement of the muscle layer. As detailed before these erosions heal rapidly and almost completely.

2. Real peptic ulcers—these are nearly always chronic with a tendency to persist and extend deeper into the muscular tissue and beyond. Attempts at healing are present but ineffective.

Etiology.—Here, again we are faced with a very difficult problem as no one factor is competent to explain all conditions. Thus several theories are current which are detailed below.

The theories are: (1) Circulatory theory, (2) Infection theory, (3) Inflammation (traumatic) theory, (4) Neurogenic theory, (5) Mechanical theory, (6) Endocrine theory, (7) Anaphylaxis theory, (8) Other theories.

(1) Circulatory Theory.—It was first believed by Virchow in 1853 who advocated that the main cause of gastroduodenal ulcer is due to circulatory disturbances which produce local devitalization of the mucosa with subsequent digestion of the part with production of ulcers. The normal living cells of the mucous membrane will not be acted on by gastric secretion and so no ulcer formation takes place but the mucosa becomes very vulnerable when the vitality is lowered by any reason and is acted on by gastric secretion. This lowering of vitality producing erosions and then ulcerations was supposed to be entirely due to vascular disturbances in the form of infarction of the terminal blood vessel supplying the portion of the mucosa affected.

Local or general circulatory disturbances, *e.g.*, thrombotic or embolic processes etc. may explain a certain number of gastric ulcers but there are many cases of arteriosclerosis or valvular disease of the heart which never show a gastric ulcer throughout life, even when hyperacidity is present. Experimentally it has been found that the nervous influence in the form of vascular spasms plays a role.

The presence of hyper-secretion of the gastric juice more specially of the hydrochloric acid combined with a low secretion of protective mucus, has given further support to this theory. That HCl plays the important role is believed to be due to the presence of these ulcerations mainly in those parts of the alimentary tract which are exposed to the action of the hydrochloric acid, and their absence from those parts which are less exposed, *e.g.*, the œsophagus, cardiac portion of the stomach and the lower portion of the duodenum, below the opening of the pancreatic duct The occurrence of marginal ulcers following gastrojejunostomy for gastric ulcer and their absence in case of similar operation following gastric cancer also give ample support to the above theory.

(2) Infection Theory.—The experimental works of Rosenow have led to a great advance in the knowledge of the pathogenesis of gastric ulcer. These experimental findings and the clinical experience definitely show that infection about the jaws, cranial sinuses and tonsil etc. are the foci from which streptococci gain access to the submucosa of the stomach causing thrombosis or embolism of the vessels resulting in hæmorrhage into the tissue by the gastric juice The streptococci isolated from the teeth or tonsil have a special selective affinity for the mucous membrane of the stomach. Rosenow could recover the streptococcus from these experimental lesions and when re-injected in other animals produced ulcers again. It is also curious to find that these experimental ulcers resembled those seen in human beings as regards the site, macroscopic appearance and also in their tendency of remaining chronic.

The normal healing power of the gastric mucosa cannot come in force on account of the persistence of the micro-organisms. This explains the difficulty of the cure of gastric ulcer.

The presence of hyperacidity and hypersecretion may merely accompany the underlying cause of gastric ulcer.

Oidium albicans has also been attributed to be one of the main causes for the chronicity of gastric ulcer. This theory has very few supporters.

(3) Inflammation Theory.—The fact that nearly all cases of gastroduodenal ulcers show gastritis or duodenitis has claimed to the origin of the inflammatory theory. It has thus been supposed that the gastric juice which has no proteolytic action upon the normal mucous membrane may have such an action on the mucosa which is already inflamed. This proteolytic action is also favoured by the hypermotility and hyperacidity of the stomach.

(4) Neurogenic Theory.—Von Bergmann believed that the hyperirritability of the antagonistic nerves vagus and the sympathetic leads to "disharmony" and this is responsible for the disturbance of the secretory and motor functions of the stomach. This hyper-irritability may be primary or may be secondary as Roessle has pointed out that the pathological condition of gall bladder, appendix or female adnexa may be the suorce of nerve impulses. But once the ulcer is formed this may itself be the source of the irritation, thus explaining the chronicity and lack of healing tendency of the ulcers.

It has been observed that the gastric ulcer patients are either "vagotonics" or "sympatheticotonics." The vagotonics show hypermotility, hyperperistalsis and hypertonicity of the stomach along with hyperacidity, bradycardia and narrow pupils. The sympatheticotonics, on the other hand, are those showing increased vascularization of the thyroid gland, exophthalmos and wide pupils. But general clinical experience does not always corroborate the classification of patients suffering from gastric ulcer into vagotonics and sympatheticotonics, as suggested by v. Bergmann.

(5) Mechanical Theory (also called the *Motor Functional Theory*).—This theory was sponsored by Aschoff and rests mainly on his conception of the function of the so-called "magenstrasse"—the gastric pathway—and of the isthmus portion of the stomach.

(6) Endocrine Theory.—It has been found experimentally that removal of endocrine gland especially the adrenals predispose to the formation of gastric ulcer. These evidences suggest the existence of some relationship between innervation and the action of the endocrine glands

(7) Anaphylaxis Theory.—Recently Ivy and Shapiro report the experimental production of acute gastric ulcer in dogs and rabbits by administration of protein injection and regards the formation of ulcer as due to anaphylaxis. Experimental feeding with various foreign proteins has also led to the production of gastric ulcer.

(8) Other Theories.—Kohler, in 1923, advocated that due to the decline of "antipepsin" in the blood serum the mucosa loses the resistance to peptic activity and this becomes responsible for ulcer formation. He has, further, formulated a test for the determination of antipepsin in blood. Kohler's test for antipepsin in blood has been found to be of no diagnostic value and his main theory has not been supported by recent workers.

More recently, it has been domonstrated that there exists a hereditary and familial predisposition with the so-called ulcer diathesis and digestion diathesis in persons liable to suffer from various gastro-intestinal disturbances. The gastric ulcer, cholelithiasis, and appendicitis (the so-called abdominal trio) are also regarded to be in close relationship by some authors.

MORBID ANATOMY.—The ulcers are generally single, small, punched out, crater-like being broader towards the mucosa than the deeper tissue, smooth floor more or less sharp margin, no thickening of the peritoneum and showing very little or no sign of inflammation.

They are more common at the pyloric end in case of gastric ulcers and in the first part of the duodenum and situated more towards the posterior wall of the organ. They may also occur in the jejunum after gastro-jejunostomy.

Chronic ulcers often show much fibrosis resulting in the narrowing and obstruction of the pyloric orifice or production of the so-called hour-glass contraction or perigastric adhesions. In case of erosion of the blood vessels hæmorrhages may occur. Perforation, fistulous communication with the neighbouring organs, rupture into the pericardium or pleura through the diaphragm (subphrenic pyo-pneumothorax) and carcinoma may occur as complications.

CHOLERA

Morbid Anatomy.—As the disease is generally of acute onset and short duration, the body is generally well nourished.

The eyes are generally sunken; the fingers, toes, lips are also shrunken and are of dark colour. The extremities are very stiff and fixed firmly, due to rigor mortis. The rigor mortis is present all over the muscles throughout the body, only being present in lesser grade in the muscles of the face and abdomen.

Rigor mortis sets in early and persists for a long time. If death has taken place in the collapsed stage, the surface presents shrunken and livid appearance. On opening the body, all the tissues seem to be abnormally dry. The muscles are darker and firmer and some of the fibres may be seen torn off.

Serous cavities are void of any fluid and sticky to the touch, and the dryness is most marked in the peritoneal coat of the small intestine. Right auricle and systemic veins contain dark, tarry, imperfectly coagulated blood, ventricles being empty and contracted.

Lungs are generally dry and shrunken, the vessels at the base specially contain thick dark blood.

The spleen and the liver are generally congested and loaded with similar tarry blood. Besides being congested, sometimes the lymphoid tissues of the Malpighian bodies show much enlargement and often become visible by the naked eye. Gall bladder is almost always full and distended with dark green, thick bile. Although the gall bladder is nearly always found greatly enlarged and full of bile, still there is very little or no bile present in the small intestines, thus rendering the contents of the intestines rice-watery. This may suggest some obstruction in the bile duct, but on dissection of the whole channel, no obstruction is seen except some amount of congestion of the mucous membrane at its termination into the duodenum. That there is no obstruction of the bile duct is shown by the fact that before dissecting the bile duct, if the distended gall bladder is pressed, the thick bile pours into the duodenum, but it requires a considerable amount of force to press it out. This partial obstruction is mainly due to the congestion of the mucous membrane of the duodenum at the opening of the bile duct.

Cholera is essentially a disease caused by the toxin produced by the organisms inhabiting the small intestines, mainly the duodenum and jejunum. It is also surprising to note that very often the post-mortem appearance of the alimentary tract, even the small intestine itself, shows very little evidence of the drastic and violent occurrences which took place during life. The whole of the alimentary tract, specially the stomach and small intestine, may show extensive congestion of the mucous membrane even amounting to petechial hæmorrhages with great enlargements of the solitary follicles of the ilium, but it is not uncommon, rather more frequent, to find very little or no change in these structures even in a case dead of a virulent type of cholera.

Stomach and small Intestines, and mainly the upper part of the latter, may be either extremely congested or of normal appearance. This depends on the amount of vomiting and purging at the time of death, and all the appearances between these two extremes are noted. Numerous petechial hæmorrhages and thick coating of mucus are found in severe cases.

The solitary follicles in the ilium similar to those of the spleen, pharynx and tonsils are seen to be enlarged and projecting out of the mucous membrane.

Large Intestines.—These as a rule, show no changes at all, or very little, if any. Much congestion of the mucous membrane may appear, mainly in the cæcum, in those cases where hæmorrhagic stool was present with the attack. The intestines are full of rice-watery fluid due to the presence of numerous small white flakes shredding out from the epithelial lining and are generally free from bile. The reaction of the contents of the intestines is alkaline.

The above changes in cholera may be summarised thus—(*i*) there is practically no ulceration of the intestines. (*ii*) The small intestine is mainly affected, stomach less so, the large intestine showing little or no change. (*iii*) Mucous membrane of the small intestine is much congested, swollen and shows superficial necrosis of the epithelial lining which is cast off as white flakes giving the contents a milky appearance the so-called 'rice-watery' evacuations. (*iv*) Hyperplasia of the lymphoid elements of the intestine, the solitary follicles becoming prominent. (*v*) The peritoneal coat is congested, dry and feels sticky. (*vi*) The gall-bladder is enlarged and distended with thick dark-green bile. (*vii*) The kidneys are congested and show degenerative changes. (*viii*) The blood is viscid and shows increase in specific grativy.

TYPHOID ULCERS

Morbid Anatomy.—The changes in typhoid fever are mostly found in the lymphoid elements of the intestines and the mesenteric glands. In the intestines the lower part of the ileum is most commonly affected on account of the richness of lymphoid elements in this situation. The ulcers are—(*i*) Round, when the solitary follicles are affected. (*ii*) Oval, in affections of the Peyer's patches, with long axis placed longitudinally along the gut. (*iii*) Projected prominently from the mucous membrane with little depressions upon the surface. When the hyperplasia of the lymphoid elements reaches its extreme, the blood vessels are compressed, anæmic necrosis takes place resulting in the formation of sloughs which are ultimately

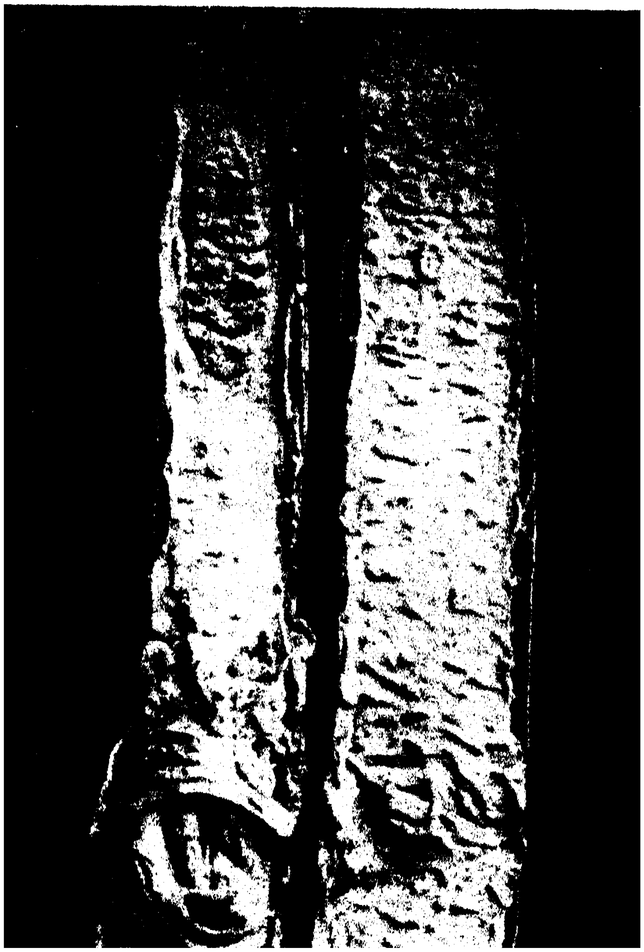


Fig. 341. Two portions of intestines showing typhoid ulcers. There is great swelling of the Peyer's patches and solitary follicles with formation of ulcers in some of them (From Path. Museum Cal. Med. Col.).

exfoliated. Fatal hæmorrhage or perforation may occur during the separation of these sloughs.

Healing takes place by the development of delicate granulation tissue with slight amount of cicatrization but is never associated with stricture of the bowel.

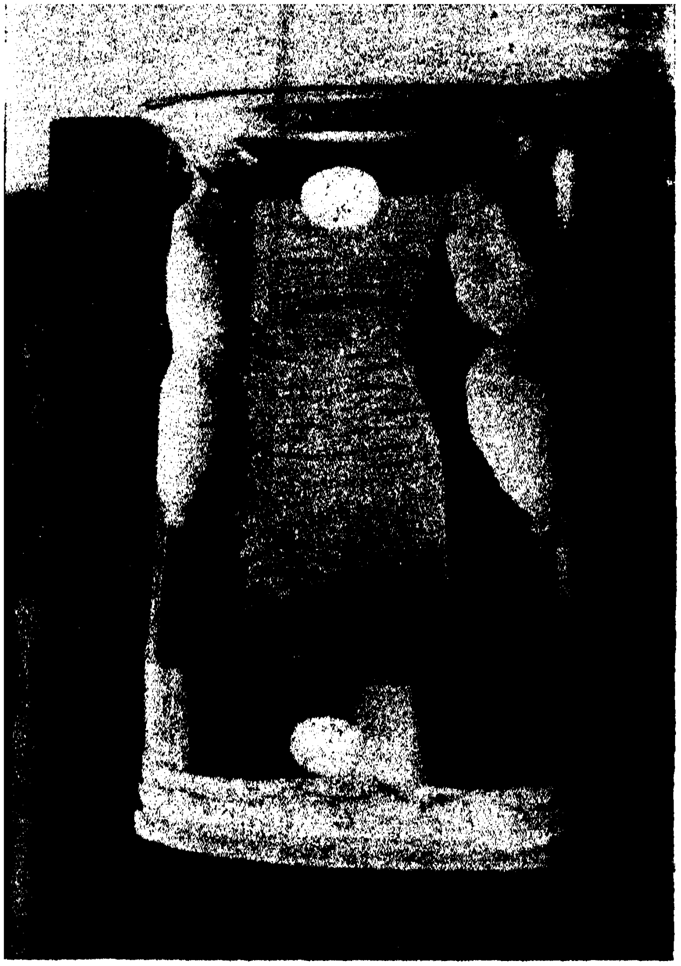


Fig. 342. Tuberculous ulcers of the intestine. The specimen showing three pieces of the intestine; the middle one being slit open to show the ulcer. The pieces on the sides showing beautifully the contraction of the gut due to fibrosis. (From Path. Museum Cal. Med. Col.).

Histology.—In the early stages there is much hyperæmia of the lymphoid follicles with subsequent proliferation of the endothelial cells of the lymphatic spaces, blood vessels and the reticulum. It is of common occurrence that these cells are proliferated to such an extent that obstruction of the lymph channels takes place, sooner or later, producing thrombosis and ultimately necrosis of the Peyer's patches. In addition to these endothelial cells number of lymphoid cells, leucocytes and plasma cells accumulate and produce the above changes. The mesenteric glands and spleen are enlarged and often show areas of focal necrosis.

TUBERCULOUS ULCERS

Morbid Anatomy.—The tuberculous affection of the intestines generally starts from the lymphoid elements either of the solitary follicles or the Peyer's patches. As the lymphatics of the gut are placed along the transverse axis and encircles it, the ulcers are also placed transversely.

Thus the ulcers are—(*i*) Mostly placed at the lower part of the ileum. (*ii*) Extend deeply and involve the submucosa, muscularis mucosæ and also the peritoneal coat. (*iii*) Placed along the transverse axis of the gut. (*iv*) Edges and base are thickened and indurated, and (*v*) Numerous tubercles are often seen on the peritoneal coat of the intestine just corresponding to the ulcers. The serous membrane is thickened and often becomes adherent to the neighbouring structures. (*vi*) In chronic cases constriction of the gut may take place due to extensive cicatrization.

Histology.—The characteristic picture of tubercle is seen, showing central caseation surrounded by giant cells, endothelial cells, round cells and fibrous tissue.

AMŒBIC DYSENTERY

Morbid Anatomy.—The lesions of amœbic dysentery are described by Rogers as follows:—" The earliest lesions are small red raised dots, which may be little larger than a pin's head. They are produced by congestion or even hæmorrhage into the mucous membrane over the early exudation into the submucous coat beneath.

As soon as a slightly larger size is reached, a light yellow spot appears in the centre of the darkly congested area, due to loss of the epithelial portion of the mucous membrane allowing the gelatinous citron-yellow-coloured infiltration of the submucous coat to appear on the surface, thus producing the earliest stage of ulceration. At this period the most marked feature is the elevation of the affected patches causing them to stand up like small buttons from the healthy mucous membrane, while the surrounding ring of congestion throws up in relief the central yellow exudation in specimens obtained within a short time after death.

The next stage is the extension of the lesions across the long axis of the bowel, the invading amœbæ meeting with least resistance along the course of the blood vessels encircling the gut in the submucous membrane.

The lesions are not continuous throughout the large bowel, even in an acute gangrenous case, for there still remain extensive areas of quite healthy mucous membrane between the enormously thickened and raised patches of tawny yellow ulceration. This is a very common and characteristic feature of amœbic dysentery.

Although the cæcum shows an extensive gangrenous ulceration involving the under surface of the ileo-cæcal valve yet the contiguous lowest parts of the ileum is quite healthy.

The ulcerated patches show the pathognomonic black cobweb-

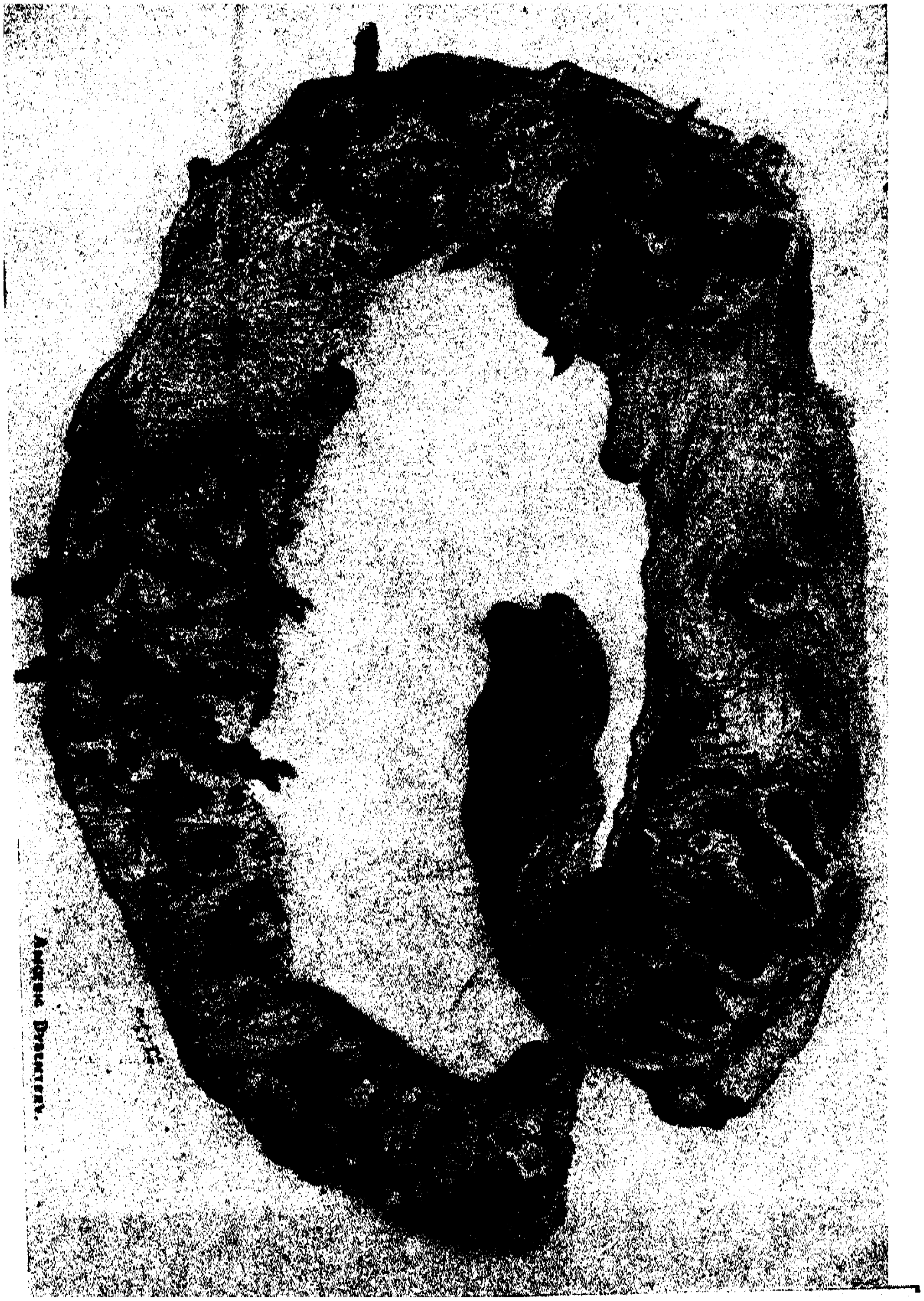


Fig. 343. Acute amœbic dysentery. (From Path. Museum Cal. Med. Col.).

like sloughs of mucous membrane of amœbic disease, produced by rapid extension of the exudation in the submucous coat, destroying the vitality of large areas of the undermined epithelial layers and causing them to be cast off as necrosed portions."

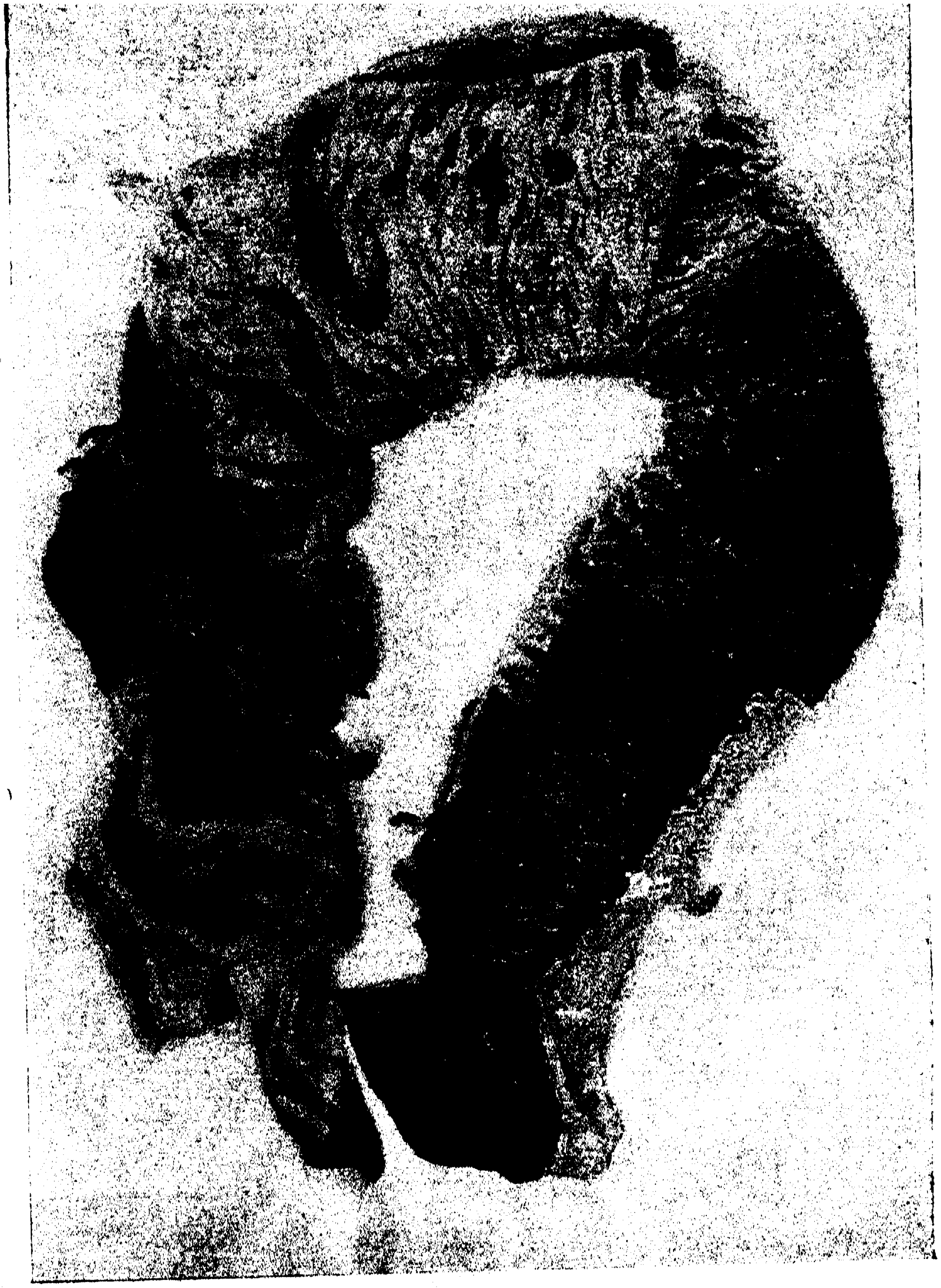


Fig. 344. Large intestine showing ulcers of bacillary dysentery. (From Path. Museum Cal. Med. Col.).

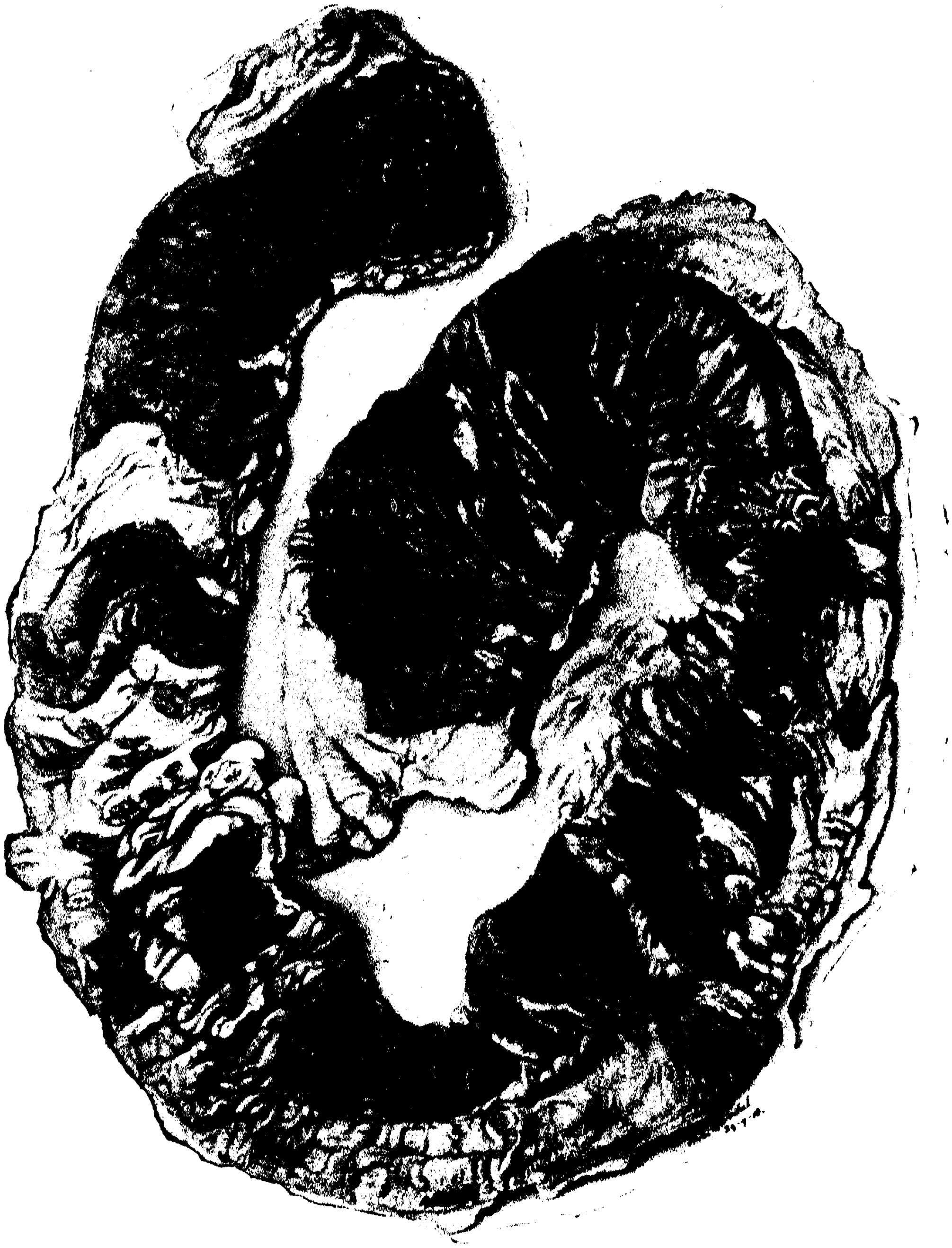
BACILLARY DYSENTERY

Morbid Anatomy.—The lesions of bacillary dysentery described by Rogers are as follows:—" *Early stages.*—In the bacillary class of dysenteries the primary lesion is essentially one of the mucous coat of the large bowel, just as that of the amœbic variety is one of the submucous layer. This difference is best seen in the early stages of the disease, which seldom come to the post-mortem room except in the very acute forms. The cæcum and the ascending colon showed the characteristic thickening due to a fibrinous granular-looking deposit in the mucous membrane, while in places, the epithelial layers had separated to leave superficial ulcers with a red, slightly-depressed base situated on a generally thickened mucosa. Scattered through the remainder of the large bowel were a number of minute yellowish white dots not much larger than a pin's head, which constitute the earliest lesions on the very surface of the mucous membrane, being inflammatory deposits in its epithelial layers.

Ulcerative stage.—The whole extent of the large bowel is involved, no extensive area of healthy mucous membrane remaining as in the case in the acute amœbic dysentery. Next it will be seen that the lowest foot or two of the ileum presents inflammatory changes of the mucous membrane similar to those in the large bowel, the disease not being limited by the ileo-cæcal valve, as is nearly always the case in amœbic dysentery. The actual lesions only differ in degree from those of the early stages above described. Thus, throughout the large bowel, as well as in the lowest part of the ileum, there is marked general thickening of the mucous membrane, with patches of greenish granular appearance, due to fibrinous deposit and necrosis of the surface epithelial layers. In addition there is very extensive serpiginous ulceration presenting slightly depressed red inflamed floors, between which portions of yellowish-white, relatively raised intact mucous membrane remain.

Chronic bacillary dysentery.—With a few exceptions the lesions are limited to the lower half or two-thirds of the large intestine. In none of the chronic cases was the lowest part of the ileum involved, as was so commonly the case in the acute series. The ulceration is usually very extensive in the affected portions of the bowel and only small tags of relatively raised intact mucosa may remain between the extensive intercurrent depressed ulcers surrounding them. If some healing has taken place, the bowel may be contracted as well as thickened."

Plate No. VIII



Acute Amœbic Dysentery

Face Page 634

CHAPTER XXXIX

CIRRHOSIS OF THE LIVER

In this affection there is a diffuse fibrosis or hyperplasia of the connective tissue of the organ, with simultaneous changes in the liver cells and capillaries.

Etiology.—It is generally due to the action of chronic irritants carried commonly by the portal circulation to the liver. Of these irritants those poduced by the entamœba histolytica seems to be the most important in this country. That alcohol plays a less important part is demonstrated by the fact that cirrhosis of the liver is not uncommon amongst Hindu widows who never touch alcohol and experimental administration of alcohol, even in very big doses and continued for a very long time, in animals, produces no cirrhosis of liver. Again, the incidence of cirrhosis of liver is seen to be rather small in percentage when a very large number of chronic alcoholics are studied. Further, Rogers observes, "A careful analysis of several thousand post mortem records convinced the writer that chronic amœbic dysentery is the most frequent cause of the liver cirrhosis, and when we recall the extensive fibrous tissue formation around large single amœbic liver abscesses, it is not surprising that chronic amœbic hepatitis should produce a general cirrhotic condition of the organ, while the combination of general cirrhosis and localised liver abscess is not very rare."

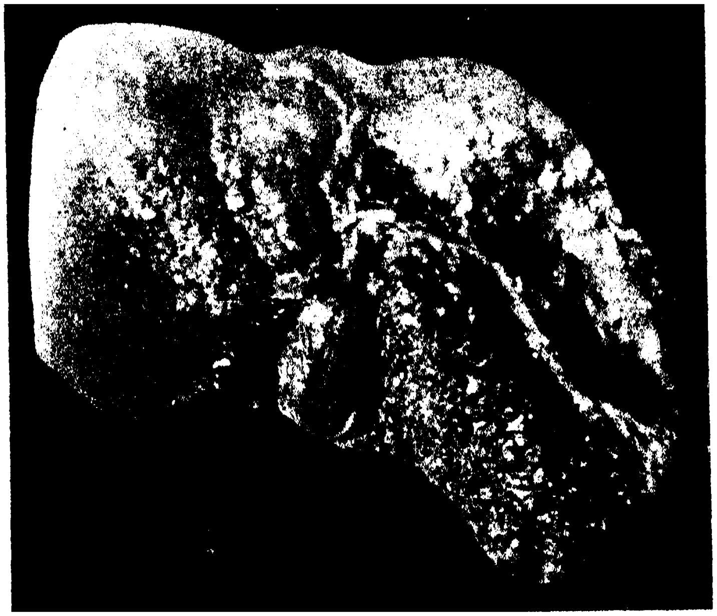


Fig. 345. Cirrhosis of liver. The under surface of the liver showing "hobnail" appearance on the surface. (From Path. Museum, Carmichael Med Col.).

The other chronic irritants may be due to the toxins produced by—(*a*) Other infective or non-infective processes carried to the liver by (*i*) The portal vein, *e.g.*, B. coli infection, over-eating. (*ii*) The hepatic artery, *e.g.*, in syphilis. (*iii*) The bile vessels, *e.g.*, in cholangitis, stasis of bile. (*b*) Accumulation of pigment, *e.g.*, in hæmochromatosis (bronzed diabetes). (*c*) Gout, rickets etc. (*d*) Experimentally, cirrhosis of liver has been successfully produced by repeated anaphylactic shocks caused by injections of protein or by injections of bacterial toxins associated with poisons which causes liver cell destruction, *e.g.*, chloroform.

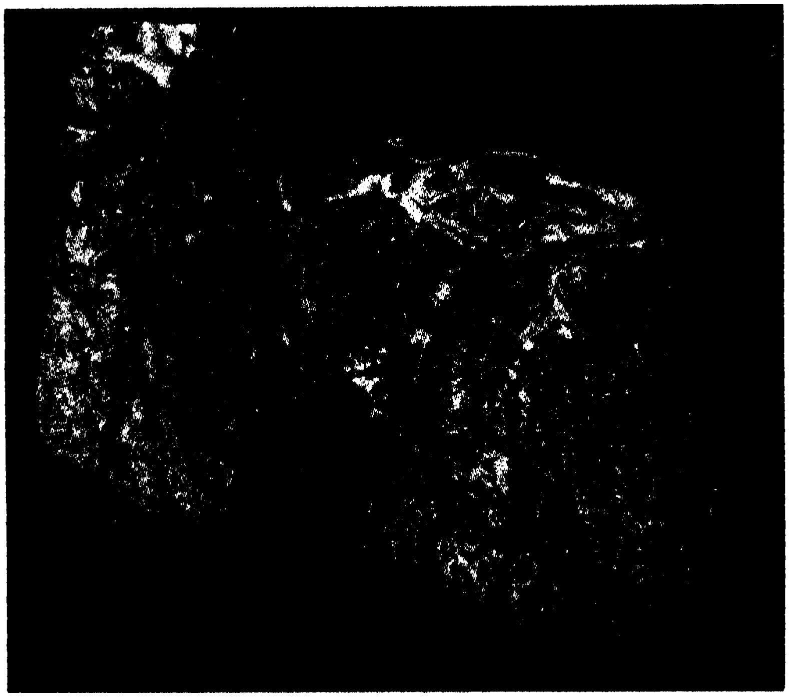


Fig. 346. Cirrhosis of liver. The under surface of the liver showing "hobnail" appearance of the surface. (From Path. Museum, Carmichæl Med. Col.)

Classification.—Due to the want of definite knowledge as regards the etiology of cirrhosis of the liver, we are placed in great difficulty in the proper classification of this condition. The provisional classification may be based on anatomical basis. This depends on the distribution of the fibrous tissue in the liver, which again depends on the following factors:—

(*a*) If the path of entry of the irritant is through the portal vein, the fibrosis is mainly found in the periportal tissue; if through the bile passages the fibrosis will mostly be found round them. (*b*) The nature of the toxic process, *e.g.*, syphilis, alcohol, etc. (*c*) The distribution of the fibrous tissue in the organ whether multi-lobular (round many lobules), uni- or mono-lobular (when round one lobule), intralobular (when inside the lobule), pericellular (when round individual cells or rows of cells), etc.

Based on the above principles, cirrhosis of the liver has been classified under the following headings:—(1) Portal cirrhosis; (2) Biliary cirrhosis (obstructive biliary cirrhosis and infective biliary cirrhosis); (3) Hanot's hypertrophic cirrhosis; (4) Pericellular cirrhosis; (5) Capsular cirrhosis (perihepatitis); (6) Pigmentary cirrhosis (hæmochromatosis); (7) Syphilitic cirrhosis.

1. Portal Cirrhosis

(*Syn.* *Atrophic cirrhosis, Lænneck's cirrhosis, multilobular cirrhosis, alcoholic cirrhosis.*)

Etiology.—Not definitely known.

Morbid Anatomy.—(*a*) In the early stages the organ is enlarged. (*b*) In the next stage, the liver shows fine granules on the surface, and the organ begins to get contracted. (*c*) In the later stage, the liver is much diminished in size and its surface becomes extremely nodular for which the term "hob-nail liver" is applied. The organ cuts with much difficulty, the consistency very firm and cannot be lacerated with firm digital pressure. The bands of fibrous tissue can be distinctly seen with the naked eye. The capsule is also thickened.

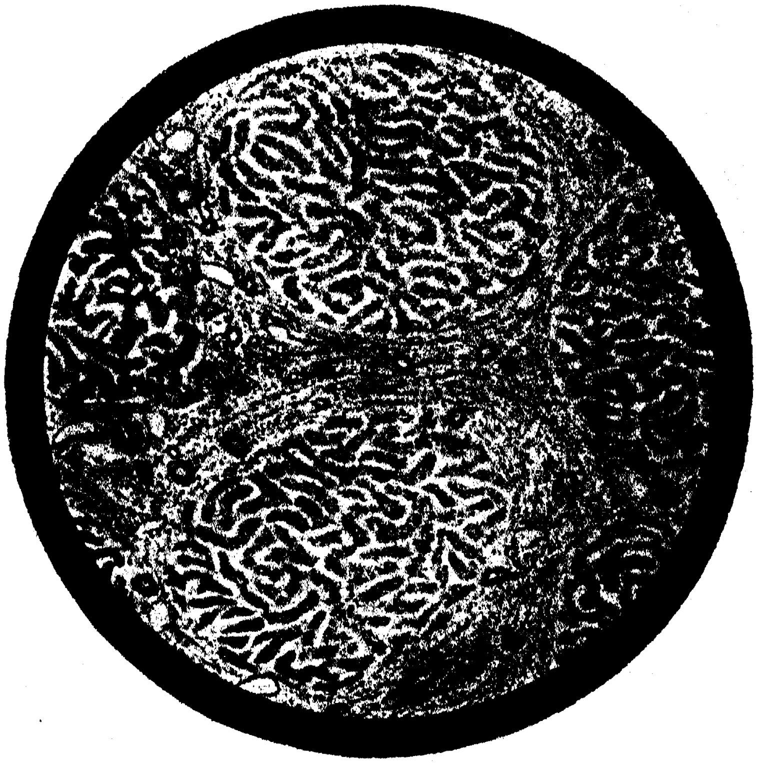


Fig. 347. Cirrhosis of liver, showing—(*i*) Great proliferation of fibrous tissue round many lobules, (*ii*) Atrophy of liver cells, (*iii*) Large number of bile capillaries, (*iv*) Round cell infiltration.

Morbid Histology.—(*a*) Formation of fibrous tissue starts in the periportal region leading to the thickening round the portal branches with no obstruction ~~of the bile duct or the hepatic~~ artery. This is illustrated by the fact that the liver after being taken out of the body can be injected with a coloured fluid, which is not possible through the portal vein.

(*b*) The bigger portal branches are affected first thus the fibrous tissue is deposited mainly in the form of big bands encircling several lobules simultaneously. This gradually extends to the smaller branches with deposition of fibrous tissue round lesser number of lobules, subsequently becoming monolobular, and ultimately entering inside the lobule to form the pericellular type.

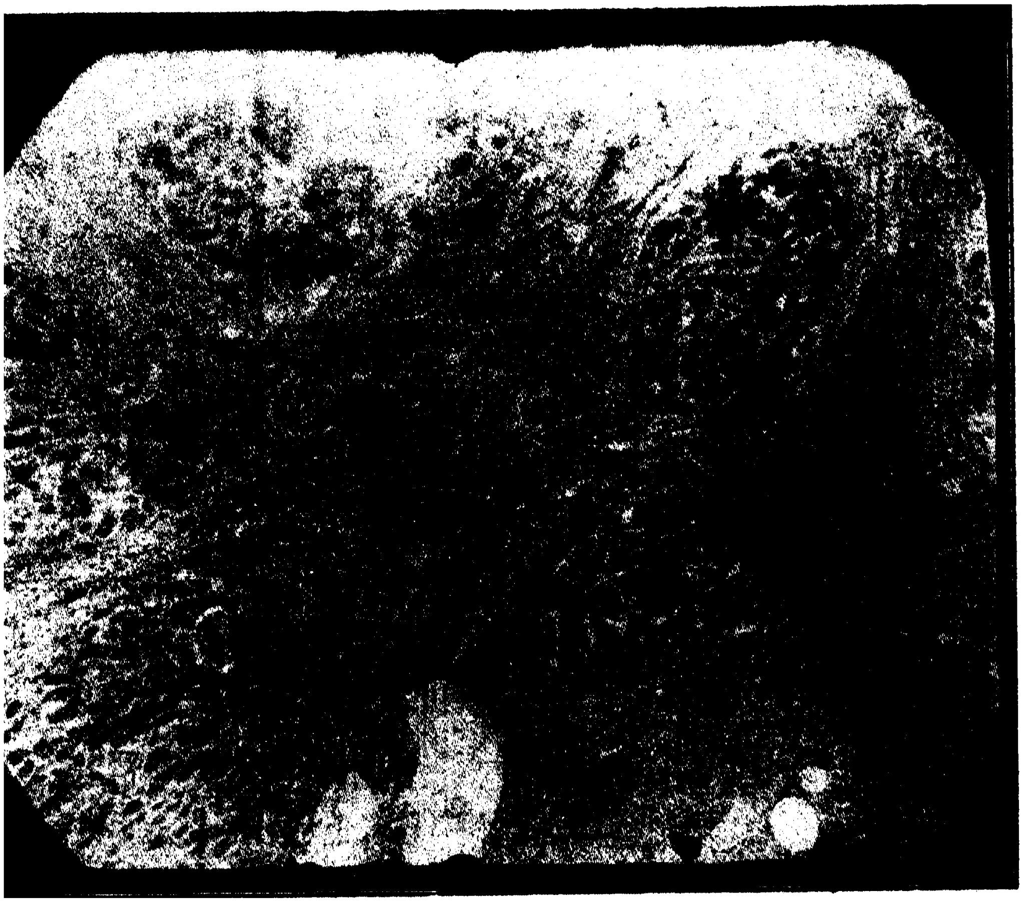


Fig. 348. Advanced stage of cirrhosis of liver showing extensive proliferation of fibrous tissue and atrophy of the liver cells.

(c) The bands of fibrous tissue are composed of fibro-connective tissue, spindle celles and round cells.

(*d*) Atrophy and fatty changes of the liver cells due to pressure of the fibrous bands.

(*e*) Presence of a large number of bile capillaries due to either (*i*) Actual proliferation of bile capillaries from the stumps of the pre-existing bile-ducts, in order to compensate for the loss of the liver cells indicating the active regeneration side by side with an extensive degeneration; (*ii*) No actual proliferation but the pre-existent bile capillaries are rendered more prominent on account of the atrophy of the liver cells; or (*iii*) That they are really not bile vessels but the atrophied liver cells looking like them.

Morbid Physiology.—These changes indicate destructive and regenerative changes of the liver cells and the types depend on the situation of the lesion. If the destructive process leaves much liver tissue to regenerate in large masses the "hobnail" type results, while regenerative changes in closely packed areas produce finer nodules. These nodules never show any definite arrangement as seen normally and no definite normal relation is present with the original portal and hepatic veins and also with bile ducts, nor even a central vein can be seen in each.

The effects are mainly those of mechanical obstruction of the portal vein and its radicles. This explains the occurrence of ascites and hæmorrhage from the stomach and intestines. Jaundice and chloæmia are generally due to the obstruction of the bile passages. There seems to be very little functional disorder, of the liver itself in spite of the profound changes in its cellular structure. It is not uncommon to find considerable accumulation of fibrous tissue with extensive degenerative change of practically all the liver cells with no functional disturbance.

2. Biliary Cirrhosis

Etiology.—(1) Obstruction to the outflow of bile, *e.g.*, in cases of stone, stricture, carcinoma, enlarged glands at the portal fissure. (2) Infection of the bile passages.

Morbid Anatomy.—In the earlier stages the liver is much enlarged, bile-stained and the surface is more or less smooth. In the later stages the organ is contracted, its surface becomes granular and its consistency very tough, yellow and bile-stained.

Morbid Histology.—(1) There is accumulation of bands of fibrous tissue round individual lobules; these bands are more slender and delicate than those of atrophic cirrhosis. (2) Increase in the number of bile capillaries throughout the fibrous bands. (3) Dilatation of the bile passages.

3. Hanot's Hypertrophic Cirrhosis

Etiology.—Unknown.

Morbid Anatomy.—(1) Liver considerably enlarged in size and weight; (2) Surface shows fine granules; (3) No much contraction of the organ as the fibrous tissue is very fine and delicate and so the pressure exerted by its contraction is insufficient to contract the liver or lead to the atrophy of its cells.

4. Pericellular Cirrhosis

Etiology.—Kala-azar, malaria, syphilis, etc.

Morbid Anatomy.—The organ is enlarged and smooth.

Morbid Histology.—The fibrous tissue accumulates round each cell and then gradually extends outwards round each lobule. The fibrous bands are very fine, hence there is little contraction of the organ.

5. Capsular Cirrhosis

In this form fibrous tissue starts from the capsule.

6. Pigmentary Cirrhosis

It is usually known as hæmochromatosis or bronzed diabetes. It contains two pigments; one containing iron, hæmosiderin; and the other containing no iron, hæmofuscin. The skin and the islets of Langerhans also suffer in addition to that of the liver.

7. Syphilitic Cirrhosis

Syphilitic affections of the liver are:—

(*i*) Cirrhosis of the liver.—(*a*) The organ may be divided into two or more pieces by big bands of fibrous tissue; (*b*) Multilobular cirrhosis; (*c*) Unilobular cirrhosis; (*d*) Pericellular cirrhosis; (*e*) Capsular cirrhosis (perihepatitis).

(*ii*) Gumma.

(*iii*) Amyloid changes.

CHAPTER XL

DISEASES OF UNKNOWN OR INDEFINITE CAUSATION

BERIBERI

This is a tropical affection of unknown causation and characterised by peripheral neuritis, œdema and cardiac dilatation. In some types, œdema may be absent. Thus two types are recognised, one, the dry type, and the other, the wet type.

There are several theories regarding the etiology of beriberi. It is generally regarded to be a condition of nutritional disorder due to deficiency of vitamin B (the anti-neuritic vitamin) from the diet. This deficiency of vitamin B is generally the result of intake of polished rice where the outer coat containing the anti-neuritic factor is removed by milling. Some regard beriberi to be due to an acute infection.

The lesions are threefold, the neuritis, the œdema and the degeneration of the heart muscle. The neuritis generally affects the peripheral nerves showing degenerative changes in the nerve endings. The vagus and the sympathetic fibres are also affected. The œdema generally affects the subcutaneous and the subserous tissues with production of hydrothorax, ascitis, hydropericardium and general anasarca. The heart is markedly dilated, more so the right chambers of the heart. The heart muscle shows fatty changes microscopically with loss of transverse striations. There is no inflammatory changes in the cardiac muscle.

EPIDEMIC DROPSY

This is closely allied to beriberi. It is characterised by gastro-intestinal disturbances, fever, rash, œdema, cardiac dilatation and tendency to hæmorrhages. The neuritis is variable. It is generally regarded to be an infective process.

PELLAGRA

Pellagra (from Italian *pelle,* meaning 'skin' and *agra,* meaning 'rough') is an endemic tropical disease of unknown causation and characterised by cutaneous rash, gastro-intestinal and nervous symptoms. There are exacerbations at recurrent intervals usually in the spring or autumn. As pellagra is common amongst the maize eating people it is supposed that maize during storage becomes overgrown with fungi, the poisonous effect of which produces pellagra. It is also been regarded as an acute protozoal infection transmitted by the fly, simulium. Others regard it as a deficiency disease.

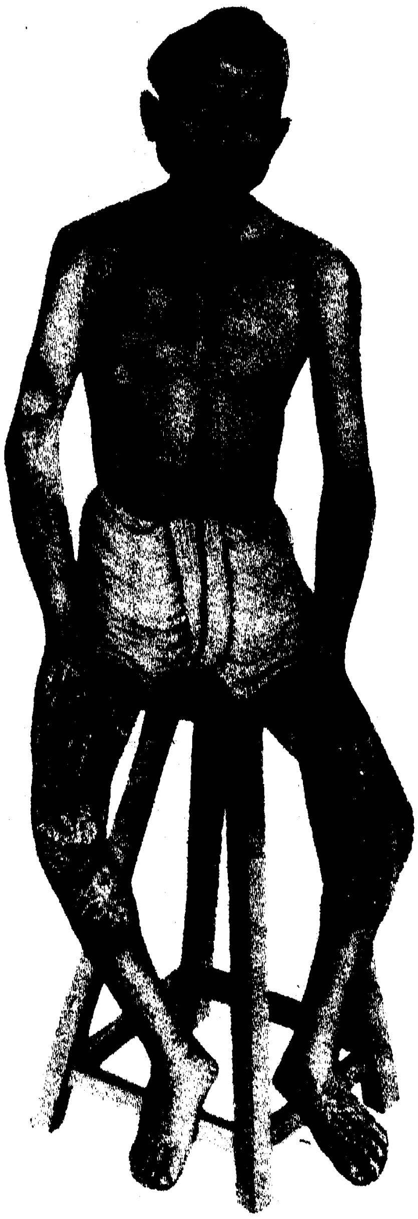


Fig. 349. Pellagra.
(From Dr. H. Ganguly.)

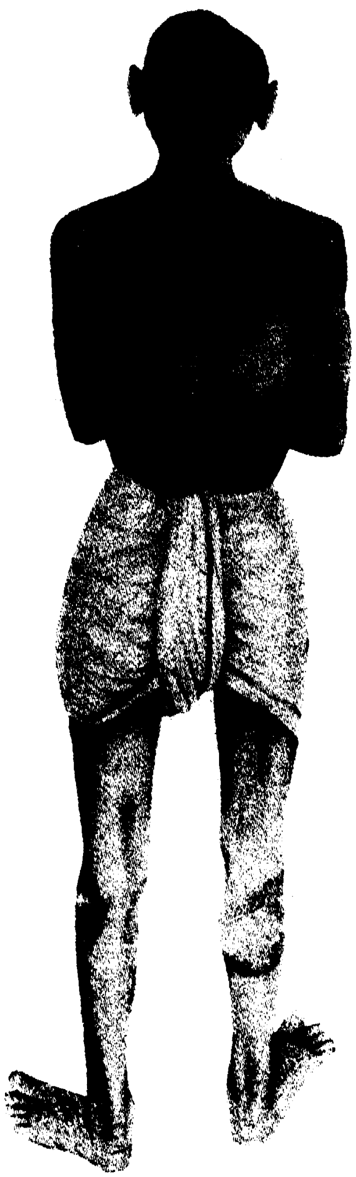


Fig. 350. Pellagra.
Back view of Fig. 349.
(From Dr. H. Ganguly.)

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TEXT BOOK OF PATHOLOGY (SECOND EDITION).

SOME PRESS OPINIONS.

The Calcutta Medical Journal

We welcome the second edition which has been thoroughly revised and rewritten and many new useful matters added. There is a respectful tribute in the preface to the famous professor P. G. Unna with whom the author had the privilege of working. The chapter on evolution of pathology is particularly welcome as it is bound to create in the students an interest in the study of pathology and inspire him to go deep into the subject. The evolution of the Indian school—the Nidanam—is highly interesting and instructive, proving clearly the very early date so far back as the seventh century A.D. in which the subject was given a shape by the Indian worker Madhaba. We congratulate the author in introducing this chapter and thus deviating from the usual course.

The subjects which cover practically all their aspects are arranged according to the needs of the students helping him to form a comprehensive idea of a disease. Diseases peculiar to our land have been, and that very rightly, specially dealt with.

Illustrations, which are mostly from Indian material, are numerous and generally well executed. The insertion of frozen sections of whole organs after Christeller's method is a welcome innovation and is an achievement which the author may be rightly proud of.

The printing and the get up of the book are quite satisfactory.

We commend the book to the attention of the students for whom it has been specially designed and the extraordinary cheapness of its price has placed it within their easy reach.

The Medical Review of Reviews

Science in all its branches has advanced with such rapid strides during the last few years that a new edition of Dr. Banerjee's Text-Book of Pathology embodying all the recent advances was urgently called for. The author is to be congratulated for bringing out the second edition of his deservedly popular book in its present form.

The book has been divided into 28 chapters and these are so arranged as to render progressive study easy both for the uninitiated and the initiated.

Of the many special features of the book one is attracted at once by the wealth of illustrations drawn from cases actually under observation of the author or one or other of his colleagues. Illustrations taken from actual cases have an intrinsic value of their own.

Again for the study of histotopography total frozen sections of whole organs have for the first time been introduced in Dr. Banerjee's book. A great advance indeed.

The laboratory hints are eminently practical and are sure to be very useful to the practical students of pathology.

In his study of bacteriology and protozoology the student is not rushed into a whirlpool to get lost but he is led step by step through the mazes of difficulty to safe quarters.

The book removes a want long felt both by the students and practitioners of this country.

One of the features of the book is that many new matters have been introduced. In fact the book has been brought up-to-date, and considering the labour bestowed on this second edition the author could have followed in the footsteps of some of the authors abroad but the good Indian teacher, as it has been his wont in olden times, has not chosen to put away from his mind the chill penury of the majority of the Indian students and has not enhanced the price of the book. The printing, etc., are excellent and there cannot be any hesitation in recommending the book to students as well as to general practitioners.

Journal of Pathology and Bacteriology

(The Official Journal of the Pathological Society of Great Britain and Ireland.)

* * * Despite some minor inaccuracies, it is obvious that a student who works through it faithfully, under guidance, will lea[illegible] a good deal of special and clinical pathology, and we can imagine that it is well adapted for use in the medical colleges of India, for which it is specially designed, and *for which European books are in many ways unsuitable.* The last sentence of the preface

can be heartily commended to the notice of the publishers of M'Callum and other successful text books: "In consideration of the well-known poverty of our students....the price has.......... been kept at Rupees twelve only."

Tropical Diseases Bulletin

(*London*)

It is not an easy task to write a work on pathology to compass the special subjects of bacteriology, protozoology, animal parasitology, histology, cytology and immunity. More difficult still must it be to present these subjects in what must, we presume, be to some extent a foreign language to the author. * * Indian students have not, as a rule, the means to spend on expensive text books. In this work they have presented a comprehensive treatment of fundamental pathological subjects which are necessary to the clear understanding of medicine and at a very moderate price. It is printed on good paper and there are numerous illustrations. * * * The illustrations are, as they ought to be, drawn from Indian sources. It is a noticeable feature of many of these that they portray advanced conditions such as are seldom met with in Western countries. Some of these illustrations are of large scale, from sections of whole organs and so thin that they admit of the use of oil immersion lenses. These sections can only be a tribute to a highly specialized technique It is generally considered a fine performance by a select few to obtain paraffin sections of whole organs with an order of thickness of 6 to 10 microns, while collodion sections are much thicker.

In the treatment of his subject the author has not by any means followed conventional lines. Some interesting chapters on the history and evolution of pathology, its mode of study and scope are introductory to the more detailed consideration of the various subjects. They stamp the work as one on a higher plane than a text book intended simply to meet the needs of examination.

Practitioner

(*London*)

This second edition of a work intended primarily for Indian students has been considerably enlarged by the addition of 150 pages and 80 figures. The preface contains an interesting and pious tribute to the author's teacher, Professor P. G. Unna, who died last year, and the first a new chapter on the evolution of pathology goes into a subject new to most readers in this country, namely the history of pathology in early Indian times; here in *Arabian** with a translation the reader is presented with an analysis of the contents of the Nidana (pathology) written by Madhaba in the seventh century A.D. The numerous subjects undertaken by this work are fairly well covered; as is natural and right, special attention is paid to the diseases prevalent in India; some of the illustrations, especially those on skin diseases and large tumours, are very graphic and remarkable. This generously illustrated text book will no doubt be much appreciated by Indian students.

The Lancet

(London)

* * * To give it any reality to medical students pathology must deal as far as is possible with the diseases which happen to be common. And looking at Dr. Banerjee's book from this point of view and considering the conditions under which it is intended to be used, we can imagine that it will continue to serve a very useful purpose. It is remarkably cheap.

Journal of American Medical Association (U. S. A.)

Pathogenic plant and animal parasites, general pathology and methods of clinical laboratory examinations are the subjects comprising this manual for medical students in India. By abridging the material, after the practice in compends, the author has set forth a large amount of information, especially in the field of tropical medicine. He worked for a time in Berlin with Erwin Christeller, whose recent death at an early age was widely lamented and at another time with Unna, the Hamburg dermatologist, also recently dead in his eightieth year. The influence of each of these investigators is evident in this book. For physicians in temperate climates the novelty of the tropical diseases illustrated compensates somewhat for the crudeness of the illustrations. In other particulars the workman[illegible] is as good as the average, or better.

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* Evidently, the reviewer means *Sanskrit*.

Archives of Pathology and Laboratory Medicine (U. S. A.)

As an outline of pathology and allied subjects, to be properly supplemented by comprehensive lectures, this text *should be very useful to students, not only in the Orient, but elsewhere.* The author has attempted to cover morphologic pathology, bacteriology, animal parasitology, laboratory methods in general and epidemiology—all in one volume. In addition, he correlates the morphologic observations in each disease condition with the pathologic physiology and the clinical picture. A few diseases are treated in more detail, especially those that are prevalent in the East, such as malaria, kala azar and the dysenteries. For the most part the author's point of view on disputed subjects is orthodox, although he has accepted the newer use of the term nephrosis to include all types of renal degeneration not accompanied by inflammatory changes. His discussion of the etiology of atherosclerosis reveals familiarity with the results of recent experimentation; in fact, most of the subjects are brought well up to date. The book may well be a valuable addition to the medical student's library.

The National Medical Journal of China

The book under review deals with pathology in its widest sense and includes under one cover the subjects of pathology, bacteriology, animal parasitology, laboratory methods and laboratory diagnosis of diseases. The author is both a pathologist and radiologist at the same time and received his special training in the German schools. In thees days of increasing specialization, these subjects are usually taught by about half a dozen different specialists, each of whom would recommend a special text book in his own field. But in Indian medical schools where qualified in these various subjects are not over-abundant, the publication of all these subjects in a single volume has met a distinct want.

KRISHNAGAR PUBLI
NOT F